CROI 2014: Basic Science Review

Mario Stevenson, PhD

In the HIV basic science categories of the 2014 Conference on Retroviruses and Opportunistic Infections, research examining obstacles to viral eradication continued to be a major component. This research encompassed areas of activity from the identification of where virus resides in individuals on suppressive antiretroviral therapy to studies aimed at eliminating long-lived viral reservoirs that persist in the face of therapy. In the area of antiviral restrictions, a number of presentations highlighted the ability of host factors to profoundly shape the interplay between virus and host and, in particular, how innate immune response opposes viral infection through the induction of antiviral restrictions.

Keywords: CROI 2014, cure, HIV, pathogenesis, restriction factors, viral reservoirs

Host Antiviral Restriction Factors

Infection of humans and of nonhuman primates is antagonized by a family of host cell proteins commonly referred to as host restriction factors, a topic discussed in a number of presentations at the 2014 Conference on Retroviruses and Opportunistic Infections (CROI), held from March 3 to 6, 2014. To date, 3 restriction factors that antagonize HIV-1 infection in humans have been identified: the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) 3 family of cytidine deaminases, tetherin (or BST-2), and SAMHD1 (sterile alpha motif [SAM] domain and histidine–aspartate [HD] domain–containing protein 1). APOBEC 3 proteins are packaged within viral particles and in the target cell, inhibit reverse transcription by a mechanism yet to be elucidated, and induce extensive G-to-A hyper mutation in viral complementary DNA (cDNA). These hypermutated genomes are biologically inactive templates for the generation of progeny virus. Tetherin is a transmembrane protein that, as its name suggests, tethers assembling virions to the surface of the virus-producing cell. These tethered variants are therefore unable to infect other cells. SAMHD1 is a deoxynucleotide triphosphate (dNTP) hydrolase that reduces intracellular dNTP levels, thereby creating suboptimal conditions for reverse transcription of viral cDNA. Given the potent antiviral activity exhibited by these cellular restrictions, why are individuals still susceptible to HIV-1 infection? The answer is that primate lentiviruses have evolved strategies to neutralize these antiviral restrictions and viral accessory proteins are central to the ability of the virus to oppose cellular restrictions. APOBEC 3 is neutralized by the viral infectivity factor (Vif) accessory protein that inactivates APOBEC 3 by inducing its proteasomal degradation. Tetherin is neutralized by the viral protein U (Vpu) protein of HIV-1. HIV-2/simian immunodeficiency virus (SIV) variants that do not encode a Vpu protein use the negative regulatory factor (Nef) protein to counteract the antiviral activity of tetherin. Most studies of how HIV-1 circumvents the action of tetherin have been in the context of Vpu and indicate that interaction of Vpu with tetherin prevents membrane association of tetherin molecules, rendering them unable to interfere with detachment of virions.

In HIV-2 and some SIV variants, the viral protein X (Vpx) protein promotes degradation of SAMHD1 in the 26S proteasome. HIV-1 viral protein R (Vpr) is the counterpart to HIV-2/SIV Vpx, and although HIV-1 infection is antagonized by SAMHD1, there is no strong evidence that HIV-1 Vpr relieves the impact of SAMHD1. Instead, HIV-1 appears to be less sensitive to the antiviral effects of SAMHD1 than HIV-2/SIV, indicating that it has evolved a different strategy with which to deal with this restriction.

In his delivery of the Bernard Fields Lecture at this year's conference, Bieniasz (Abstract 17) discussed his ongoing research on tetherin and how it counteracts viral replication. To illustrate that the antiviral action of tetherin is simply a consequence of its ability to prevent detachment of virus particles, Bieniasz assembled a synthetic tetherin molecule from the domains of unrelated proteins. The synthetic tetherin, which comprised domains from the transferrin receptor, the dystrophia myotonica protein kinase, and the urokinase plasminogen activator receptor, was very effective in inhibiting HIV-1 particle release, despite the fact that the synthetic tetherin had no sequence homology to the viral tetherin molecule. This set of experiments clearly argues that the antiviral activity of tetherin is based solely on its ability to trap viral particles on the surface of the infected cell. These experiments also demonstrate that the antiviral activity of tetherin does not require specific interaction with viral proteins.

Dr Stevenson is a Professor in the Department of Medicine and Chief of the Division of Infectious Diseases at the University of Miami Medical School in Miami, Florida. Send correspondence to Mario Stevenson, PhD, University of Miami Leonard M. Miller School of Medicine, Life Science Technology Park, 1951 NW 7th Avenue, Rm 2331B, Ste 200, Miami, Florida 33136. Received on March 18, 2014; accepted on March 19, 2014.

Bieniasz described how a better understanding of the mechanisms by which lentiviruses counteract cellular restrictions could aid in the design of HIV-1 variants that replicate in monkeys. The availability of such HIV-1 variants could improve strategies for preclinical evaluation of vaccines and new antiretroviral drugs. However, HIV-1 does not replicate in macaques because the mechanisms it has evolved to counteract antiviral restrictions in human cells are limited in their ability to oppose restrictions in simian cells. For example, HIV-1 Vif does not efficiently neutralize macaque APOBEC 3 proteins, and HIV-1 replication in macaque lymphocytes is 4 orders of magnitude less efficient than SIV replication. Bieniasz engineered an HIV-1 variant that contains SIV *vif* and replicates almost as efficiently as SIV in macaque lymphocytes. This virus has formed the basis for experiments in which HIV-1 variants containing altered envelope genes and SIV *vif* were adapted to replication in pigtail macaques. Changes appeared in viruses as they evolved to replicate more efficiently in macaques, including changes in envelope that conferred X4 tropism and changes in *vpu* that conferred the ability to neutralize tetherin. Simian-passaged HIV-1 was able to neutralize tetherin in monkeys, but it simultaneously lost the ability to neutralize tetherin in humans. This was analogous to the series of events that occurred when the HIV-1 ancestor acquired the ability to neutralize human tetherin as it entered its human host. Simian-tropic HIV-1 caused disease in pigtail macaques and recapitulated some key features of AIDS in humans. Although these studies bring us much closer to a simian model for HIV-1, additional blocks to simian-tropic HIV-1 replication in macaques need to be overcome before the adaptation of HIV-1 to its simian host is complete.

In his plenary presentation, Malim (Abstract 119) discussed the impact of type I interferon on HIV-1 infection. All pathogens contain pattern recognition receptors that sense foreign elements in RNA, DNA, and protein. These foreign elements are referred to as pathogen-associated molecular patterns. The components of HIV-1 that are sensed by host pattern recognition receptors to trigger an interferon response have been a topic of intense investigation. In myeloid cells, it is also apparent that HIV-1 escapes the detection of the innate sensing apparatus by cloaking itself. Pathogen-associated recognition receptors in the viral capsid protein are hidden from the innate sensing apparatus because capsid cloaks itself in cellular proteins to evade innate sensing. Mutations in capsid that help uncloak these motifs lead to induction of an interferon response. This theme was expanded on in Session S7. Towers (Abstract 154) described an HIV-1 capsid mutant (P90A) that, unlike its wild-type counterpart, efficiently induced interferon on infection of macrophages. Sensing of the incoming virus appeared to be triggered by viral cDNA or a reverse transcription intermediate. Towers went on to implicate cyclophilin A and cleavage and polyadenylation specific factor 6 (CPSF6) as cellular proteins that cloak capsid from DNA sensors so as to prevent activation of the interferon response. This predicts that agents that uncloak the capsid and expose it to DNA sensors could have utility as antiviral agents. To this point, Towers demonstrated that cyclosporins, which inhibit cyclophilin from binding to capsid, induced an interferon response and inhibited HIV-1 infection in monocyte-derived macrophages. Therefore, the cyclophilin-capsid interface represents a novel target for therapeutic intervention of HIV-1 infection.

There has been much attention focused on the sensors that detect nascent viral cDNA. Malim summarized research implicating cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS), an enzyme that resides in the cytosol and recognizes incoming viral cDNA. The action of this enzyme results in the generation of the cyclic dinucleotide cGAMP that binds to and activates stimulator of interferon genes (STING), leading to signaling and induction of type I interferon. However, other sensors have been implicated in the recognition of viral elements, including the recognition of cDNA by host nucleases such as three prime repair exonuclease 1 (TREX1) and the recognition of virion RNA by Toll-like receptor 7 (TLR7). An important question is the extent to which avoidance of DNA-sensing elements impacts HIV-1 infection of T cells.

Identification of host cell factors that are induced by interferon and that mediate the antiviral activity of the interferon response has also been of intense interest. In Abstract 46, Kane and colleagues described research implicating myxovirus resistance 2 (MX2) as an interferon-induced inhibitor of HIV-1 infection. MX proteins are interferon-induced, dynamin-like guanosine triphosphatases (GTPases) that inhibit infection of a wide variety of viruses including influenza A virus, measles virus, hepatitis B virus, and vesicular stomatitis virus. MX2 was identified as an inhibitor of HIV-1 replication by comparing gene expression profiles in cell lines that differed in their ability to support the inhibitory action of interferon at the early stages of HIV-1 infection.[1] MX2 was found to act at a stage that followed reverse transcription but preceded nuclear import of viral cDNA. MX2 appears to be required for full antiviral activity of interferon against HIV-1, and neutralization of MX2 using short hairpin RNAs (shRNAs) was sufficient to nullify the inhibitory action of interferon treatment on HIV-1 infection of THP-1 cells. Nuclear localization of MX2 was required for the antiviral action of MX2 and the antiviral activity of MX2 was enhanced when target cell cycles were arrested, suggesting that MX2 prevents the nuclear localization of viral DNA. Furthermore, the antiviral activity of MX2 was mediated by the HIV-1 capsid protein, as some capsid point mutants were insensitive to MX2 inhibition.

Borrow (Abstract 157) discussed how type I interferon response may impact HIV-1 transmission. The majority of heterosexual transmissions is established by a single transmitted founder virus, despite the fact that there is a highly diverse population of viruses in the donor. This suggests either the presence of a barrier that restricts

transmission of the virus or that there are factors within the recipient that restrict the establishment of transmitted viruses. Borrow and colleagues have pursued the hypothesis that innate immune responses mediated by interferons, for example, may exert selective pressure on the transmitted virus. This would seem to predict that interferon-mediated pressure may drive the emergence of interferon-resistant viruses in the acute phase of infection. Primary HIV-1 isolates were generated from cryopreserved plasma by expansion in primary CD4+ T cells and then examined for sensitivity to interferons. HIV-1 isolates derived from acutely infected subjects were found to be more resistant to interferon than viruses obtained from the same patients at the chronic stage of infection. Type I interferon resistance was found to decline rapidly following acute infection and viruses obtained 6 months from the onset of symptoms were found to be substantially less resistant to interferon than viruses obtained in the acute phase of infection. Borrow went on to examine whether the establishment of HIV-1 infection by interferon-resistant viruses was caused by selection of interferon-resistant variants from the donor quasispecies or whether there was preferential transmission of interferon-resistant viruses that preexisted in the donor quasispecies population. Analysis of viruses obtained from an acute-to-acute transmission pair indicated that interferon-resistant viruses in the donor viral quasispecies population were selectively transmitted, supporting the notion that type I interferons contribute to the transmission bottleneck. Viral determinants that mediate the differential resistance to type I interferon are under investigation.

Viral Reservoirs and Cure Research

Many sessions and presentations at CROI dealt with issues related to understanding obstacles to viral eradiation and attempts to achieve viral eradication in HIV-infected individuals. The scientific rationale for pursuing viral eradication in HIV-1–infected individuals derives from the Berlin patient, Timothy Brown, who in 2007 received a CC chemokine receptor 5 (CCR5)-Δ32 stem cell transplant and has since had undetectable levels of HIV-1 RNA.[2] The stem cell transplant was necessitated after Brown developed acute myelogenous leukemia. He received an allogeneic transplant from a related donor who carried a homozygous deletion in the CCR5 gene. Many questions have arisen as to how this course of treatment cured Brown and whether this feat could be recreated. Henrich (Abstract 144LB) discussed 2 patients who received allogeneic stem cell transplants, but in each case the donors were heterozygous for the CCR5-Δ32 mutation. There was complete chimerism, with less than 0.001% of host peripheral blood mononuclear cells (PBMCs) persisting after transplant. Following transplant, 1 subject remained on antiretroviral therapy for 2.8 years and the other remained on therapy for 4.5 years, during which time proviral DNA levels in PBMCs and HIV-1 RNA levels in plasma remained undetectable. Encouraged by these results, the investigators interrupted the subjects' therapy; viral rebound occurred at 3 months and 8 months, respectively, after cessation of antiretroviral therapy. Therefore, despite a 3 $\log_{10}$ copies/mL to 4 $\log_{10}$ copies/mL reduction in viral reservoir size, allogeneic transplantation with heterozygous CCR5-Δ32 cells did not eliminate all viral reservoirs. One possible explanation is that the reservoirs that maintained HIV-1 in these individuals comprised cells that were long-lived or did not circulate in blood. Drawing conclusions about the source of the virus that persisted in these patients is difficult because of sampling issues with methodologies used to identify infected cells. Sampling of gut and peripheral blood failed to reveal the presence of viral DNA in different memory CD4+ T cell subsets. This begs the question of whether there may be non-CD4+ T cell reservoirs of HIV-1 in individuals on suppressive antiretroviral therapy.

Several presentations offered intriguing evidence that HIV-1 may reside in cells other than CD4+ lymphocytes in aviremic individuals. Wong and colleagues (Session O-12, Abstract 137) examined the distribution of viral DNA in CD4+ T cell subsets in the gut, lymph nodes, and blood of 8 individuals on suppressive antiretroviral therapy. The study subjects underwent leukapheresis and lymph node and rectal biopsy. Cells were separated into naive, central, transitional, and effector memory subpopulations followed by analysis of viral DNA and RNA. Viral distribution was found to be tissue dependent. Viral DNA was most abundant in effector memory cells in rectal tissue, and most of the viral DNA in blood was found in central memory and transitional memory cells. However, levels of viral DNA in cell samples always exceeded the amount that could be accounted for in CD4+ T cell subsets. In cells obtained from rectal tissue, levels of viral DNA in non-CD4+ T cells approached those found in CD4+ T cells. The investigators went on to demonstrate that these cells were macrophages. Although contamination of macrophage cell populations with CD4+ T cells is always a concern in these experiments, the frequencies of viral DNA in macrophages made it unlikely that the signals were caused by contaminating T cells. Abstract 410 described the identification of viral DNA in alveolar macrophages. Of the 22 HIV-1–infected subjects studied, 82% had undetectable plasma HIV-1 RNA levels. Viral DNA was detected in 78% of individuals with undetectable HIV-1 RNA levels, and macrophages from these individuals exhibited impaired phagocytic properties. The half-life of resident tissue macrophages, especially following HIV-1 infection, is relevant to the understanding of how HIV-1 persists in individuals on long-term therapy.

Lichterfeld (Abstract 54) presented evidence for a stem cell reservoir of HIV-1. Stem cells exhibit the greatest longevity due to their ability to undergo cell renewal and homeostatic proliferation, and their ability to resist apoptosis. Lichterfeld presented evidence that CD4+ T memory stem cells are susceptible to HIV-1 infection and harbor high levels of viral DNA

in infected individuals on suppressive antiretroviral therapy. Viral DNA levels in these CD4+ T memory stem cells were stable over long-term suppressive therapy. It remains to be determined whether the virus present in this cell population is biologically active and is able to drive viral recrudescence in individuals whose therapy is interrupted. Maintenance of viral DNA in stem cells is likely to be a consequence of the intrinsic stability of this cell population. In addition, self-renewal of stem cells followed by duplication of proviruses during mitosis would contribute to maintenance of this reservoir.

Homeostatic proliferation has also been implicated as a mechanism through which the latent CD4+ T cell reservoir is maintained.[3] Evidence for homeostatic maintenance of viral reservoirs is provided indirectly by the nature of viruses that rebound following years of suppressive antiretroviral therapy in which the rebounding population has identical viral sequences. This is most likely explained by recrudescence of virus from identical proviruses that were generated through numerous rounds of mitosis. Abstracts 138 and 407LB examined integration sites in individuals on suppressive antiretroviral therapy in order to distinguish whether emergence of identical sequences in individuals whose antiretroviral therapy was interrupted was due to expansion of a population of identical proviruses through homeostatic proliferation, or the rapid outgrowth of a single virus variant with a high fitness advantage.

Hughes (Abstract 407LB) presented evidence that as many as 50% of the integration sites in individuals on suppressive antiretroviral therapy were a result of clonal proviral expansion. Furthermore, the nature of the integration sites indicated that there was some selective pressure exerted by the genetic location of the integration site. Therefore, some integration sites may impact the proliferation and survival of the host cell and promote expansion of that provirus; this would seem counterintuitive as HIV-1 is well recognized for its cytopathic effect on the host cell. It is possible that some expanded proviruses are defective and have little impact on host cell function. Although some duplicated proviruses are clearly capable of driving the synthesis of virions, as evidenced by their appearance in plasma following treatment interruption, it is not known whether proviruses maintained by homeostatic proliferation or by integration site–driven cell proliferation can serve as templates for biologically active viruses that can fuel viral recrudescence in individuals whose therapy is interrupted.

Key elements in the strategy for eradication of long-lived viral reservoirs in HIV-1–infected patients on suppressive antiretroviral therapy include reactivation of viral gene expression and elimination of the reactivated cell through viral cytopathic effects or immune-mediated clearance. Because viral latency is considered to be maintained by the state of chromatin architecture, agents that modify chromatin have been explored for their ability to reactivate latent virus in cellular models of latency in vitro and in HIV-1–infected individuals. Although chromatin modifiers such as histone deacetylase (HDAC) inhibitors can reactivate latent HIV-1 in cell line–based models of viral latency, evidence that these agents can induce HIV-1 in infected individuals on suppressive therapy is lacking. Session P-F9 featured several presentations examining the impact of chromatin-modifying agents on viral and cellular gene expression. Abstract 438LB demonstrated that panobinostat was able to induce HIV-1 transcription as well as plasma viremia in patients on suppressive antiretroviral therapy. In latently infected cell lines, panobinostat exhibited far greater potency than other HDAC inhibitors, such as belinostat or vorinostat. Those findings prompted exploration of panobinostat in HIV-1–infected individuals. The treatment was found to be well tolerated and repeated cycles of panobinostat resulted in episodic induction of viremia.

Results were less encouraging with repeated doses of vorinostat (Abstract 435LB). As has been demonstrated by the Margolis group,[4] a single dose of vorinostat induced cell-associated HIV-1 RNA levels in quiescent T cells from HIV-1–infected individuals on suppressive antiretroviral therapy. However, when vorinostat was administered in weekly cycles, there were limited increases in cell-associated viral RNA and these only occurred in 3 of 5 individuals. In addition, changes in histone acetylation were less apparent following repeated cycles of vorinostat. Therefore, vorinostat may have the capacity to reactivate HIV-1 following a single dose but repeated doses lead to a blunted response. It is unclear whether blunted responses following repeat dosing will apply to other, more potent HDAC inhibitors.

Immunopathogenesis

Pathogenic lentivirus infection, such as HIV-1 infection in humans, is distinguishable from nonpathogenic infection, such as SIV infection of sooty mangabeys (SIVsm), by the extent of immune activation. In pathogenic lentivirus infections, there is chronic immune inflammation and this undermines immune homeostasis and likely drives viral persistence. Immune inflammation is associated with disruption of the gastrointestinal epithelial barrier, and this leads to translocation of microbial products, which contributes to immune inflammation and drives AIDS progression. T helper 17 (T_H17) cells are important for maintenance of the epithelial barrier and production of cytokines, such as interleukin (IL)-17 and IL-21, and antimicrobial agents, such as defensins. T_H17 cells are susceptible to HIV-1 infection and there is a depletion of this cell population in HIV-1–infected individuals.

Abstract 77 presented evidence that early initiation of antiretroviral therapy minimized damage to the mucosal barrier and reduced subsequent T cell activation. Individuals who initiated antiretroviral therapy at Fiebig stages I or II had statistically significantly higher levels of T_H17 cells, similar to levels seen in HIV-1–uninfected controls. At 6 months and 24 months post-treatment initiation, subjects at Fiebig stages I or II maintained low levels of

immune activation that were similar to those seen in HIV-seronegative individuals. Recent studies suggest that dysbiosis of commensal microbiota interferes with immunologic reconstitution following antiretroviral treatment. Oral probiotics have previously been shown to increase the extent of gut CD4+ T cell reconstitution but not T_H17 recovery in nonhuman primates treated with antiretroviral therapy. Abstract 83 examined the impact of IL-21 administration in SIV-infected macaques receiving probiotic supplementation. The investigators observed that probiotic and IL-21 supplementation of antiretroviral therapy in SIV-infected macaques led to CD4+ T cell reconstitution and increased T_H17 frequency but was not associated with increased viral load. This study has important implications for strategies aimed at reversing the damage to the mucosal epithelium by HIV-1 and at improving immune reconstitution during antiretroviral therapy.

Echoing this theme, Abstract 315 explored alterations in gut microflora in HIV-1–infected individuals and whether this was influenced by antiretroviral treatment. Gut microbiota was altered during HIV-1 infection and correlated with immune status. Abstract 317 discussed research aimed at identifying translocating bacteria in SIV-infected macaques. The bacterial population was identified through 454 sequencing of 16S ribosomal DNA. Proteobacteria were found to preferentially translocate from the gastrointestinal tract in SIV-infected macaques. Because some of the translocating bacteria were motile and flagellated, it is likely that these pathogenic species contribute to immune activation. These studies provide the rationale for monitoring interventions that maintain a healthy gut microbiome, reduce immune activation, and improve disease prognosis.

Financial affiliations in the past 12 months: Dr Stevenson has no relevant financial affiliations to disclose.

A list of all cited abstracts appears on pages 632-638 of this issue. Abstracts are published in *Top Antivir Med.* 2014;22(e-1):1-570, a special online issue available at www.iasusa.org/pub.

Additional References

1. Kane M, Yadav SS, Bitzegeio J, et al. MX2 is an interferon-induced inhibitor of HIV-1 infection. *Nature*. 2013;502(7472):563-566.
2. Hutter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med.* 2009;360(7):692-698.
3. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med.* 2009;15(8):893-900.
4. Archin NM, Liberty AL, Kashuba AD, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretro viral therapy. *Nature*. 2012;487(7408):482-485.

Top Antivir Med. 2014;22(2):574-578