Review of Basic Science Advances in HIV

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The Conference on Retroviruses and Opportunistic Infections (CROI) is held annually to provide a forum for scientists to hear the most recent advances in the field of HIV and AIDS research. Although the conference has a primary mission to showcase advances in the prevention and management of HIV-1 infection and opportunistic infections (in particular, tuberculosis and hepatitis C virus [HCV]), there continues to be a strong basic research component. Research on cellular factors that influence the interplay between the virus and the host cell, and especially, cellular factors that antagonize viral replication, had the greatest presence at the conference. In the area of retroviral pathogenesis, research on viral reservoirs and mechanisms of viral persistence in the face of antiretroviral therapy generated considerable interest. Research on mechanisms of viral persistence is beginning to reveal strategies, some of which were the focus of presentations at CROI, to eliminate long-lived viral reservoirs.

Cellular Restrictions

In his plenary lecture, Emerman discussed how primate lentiviruses, including HIV-1 and HIV-2/simian immunodeficiency virus (SIV), have been shaped through evolutionary conflict with their primate hosts (Abstract 19). In the last century, HIV-1 has been acquired from chimpanzees at least 4 times and humans have acquired HIV-2 at least 8 times from sooty mangabeys. Therefore, Emerman focused his discussion on why humans are infected with some lentiviruses and not others, and how lentiviruses that infect humans have adapted to their human hosts.

Emerman's research has focused on ancient viral pathogens and how their antiviral defenses deal with modern viruses. These antiviral defenses are proteins that are encoded by primates. These include APOBEC 3, SAMHD 1, and tetherin. APOBEC 3 and SAMHD 1 act on the reverse transcription step of viral replication, but at different levels. APOBEC 3 deaminates viral complementary DNA (cDNA), rendering it incompetent to serve as a template for production of functional viral transcripts and proteins. SAMHD 1 is a nuclear protein that shuttles into the cytoplasm and reduces nucleotide pools to render the cell poorly permissive for reverse transcription.

Tetherin acts at the site of viral budding and interferes with the detachment of the maturing virus particle from the surface of the infected cell. Viruses have evolved evasion strategies to circumvent cellular defenses such as tetherin and establish infection within the host cell. Emerman used the "arms race" as an analogy to describe the conflict between the antiviral factor and the virus-encoded antagonist. This conflict results in rapid evolution of both cellular defense factors and the viral antagonists of those factors. Over generations, host escape from the viral antagonist essentially neutralizes the ability of the virus to protect itself from the host-encoded defense factor. This forces a viral readaptation that allows the viral antagonist to once again neutralize the host defense factor.

In the absence of viral infection, the antiviral gene does not acquire mutations because it is not under selective pressure. However, the presence of a new virus forces the accumulation of mutations that influence the interaction with the viral antagonist. This process, by which viral infection drives polymorphisms within cellular genes, is referred to as positive selection. Emerman described studies that cloned genes from primate lentiviruses and antiviral genes representing 30 million years of viral and antiviral evolution. These viral and cellular genes were then subjected to functional analysis including antiviral activity and ability to neutralize cellular defense proteins. This analysis allowed an estimation of when the virus entered the primate lineage, how acquisition of an antiviral gene influenced the fitness of the host, and how the virus evolved to adapt to its new host. Using APOBEC 3DE, which is poorly active against HIV-1, as an example, Emerman was able to determine the timing of an ancient viral infection occurring between 2 million to 5 million years ago. The positive selection of host antiviral genes can be used to gauge whether a virus is imparting some fitness cost on the host. For example, although SIV from African green monkeys (SIV_{agm}) is considered nonpathogenic, the ongoing adaption between APOBEC 3G and SIV Vif indicates that the virus is not benign in its host and that there is a fitness disadvantage as a consequence of the infection.

Finally, Emerman discussed how his evolutionary analysis can be used to provide insight into the adaption of a virus to a new host. For example, HIV-1 Vpu antagonizes the antiviral action of tetherin. Chimpanzee SIV (SIV_{cpz}), the immediate ancestor of HIV-1, encodes a Vpu protein that does not counteract tetherin; other SIV lineages, including sooty mangabey SIV (SIV_{sm}), do not contain Vpu, but counteract tetherin via the Nef accessory protein. Although SIV Nef could counteract tetherin in chimpanzees, on transfer to humans, SIV Nef was unable to neutralize human tetherin—the site of its interaction had been deleted during human evolution. Therefore, HIV-1 altered Vpu in the transmembrane domain so that SIV

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Nef could interact with and neutralize tetherin. This occurred only in pandemic (group M) HIV-1 and not in nonpandemic (group O) HIV-1, indicating that the current epidemic may be explained in part by changes in the transmembrane domain of Vpu that allow HIV-1 to neutralize tetherin.

Viruses adapt to the new host not only by evolving a new function within an existing gene (such as the case with vpu and HIV-1) but also by evolving a new viral gene to combat a host restriction. HIV-2, macaque SIV (SIV_{mac}), and SIV_{sm} harbor a *vpx* gene that is not contained within HIV-1 or SIV_{cpz} or gorilla SIV (SIV_{gor}) lineages. Recently, *vpx* has been demonstrated to degrade a cellular antagonist called SAMHD 1. SAMHD 1 limits infection of myeloid cells (dendritic cells and monocytes) by depleting deoxynucleotide triphosphate (dNTP) pools, thereby limiting reverse transcription of the virus. Although HIV-2 and SIV_{sm} lineages encode a Vpx, HIV-1 does not. Therefore, in the ancestral primate lentivirus that gave rise to the HIV-1 and HIV-2 predecessors, the ancestral *vpr* gene acquired the ability to degrade SAMHD 1.

Further along the evolutionary pathway, the HIV-2 predecessor acquired a new gene that specialized in its ability to degrade SAMHD 1. Although no Vpx-like activity has so far been detected in HIV-1, this virus retains the ability to infect macrophage without the apparent ability to degrade SAMHD 1. In summary, the conflict between lentiviruses and their hosts involves antagonism of viral infection by cellular defenses and neutralization of those defenses by viral accessory proteins. Viruses adapt to selective evolution of host cell defenses by acquiring a new gene with specialized ability to counteract the host defense or by adapting to be able to neutralize the cellular defense.

Research in the area of antiviral restrictions has accelerated in pace with the recent identification of a novel antiviral restriction that selectively acts to block viral reverse restriction in myeloid-lineage cells. In the past year, the research groups of Benkirane[1] and Skowronski[2] independently identified SAMHD1 as the cellular target of the Vpx protein encoded by HIV-2 and most SIV. As discussed in the symposium on host cell factors, SAMHD 1 is a newly discovered antiviral restriction that specifically antagonizes lentivirus replication in myeloid cells including monocytes and dendritic cells (Abstract 63). In the absence of Vpx, infection of primary lymphocytes and T-cell lines is not affected. However, infection of these myeloid cells is absolutely dependent on a functional Vpx and in its absence, viral replication is blocked at the reverse transcription step.

Vpx (like Vpr) is packaged within virions. Therefore, it is clear that the Vpr and Vpx proteins act at an early stage in viral replication before de novo synthesis of viral proteins. In earlier work by Sharova and colleagues[3] heterokaryon analysis in which permissive HeLa cells were fused with nonpermissive macrophages indicated that Vpx was counteracting a dominant-negative restriction. This research provided the impetus for studies to identify the Vpx-associated restriction. SAMHD 1 appears to have biologic properties that underscore the belief that it is the restriction targeted by Vpx. For example, SAMHD 1 is degraded in the proteasome in the presence of Vpx. Silencing of SAMHD 1 increases infection of nonpermissive cells. Expression of SAMHD 1 in permissive cells rendered them nonpermissive to HIV-1 infection. SAMHD 1 is a deoxynucleoside triphosphate triphosphohydrolase and mutations in this gene have previously been shown to be associated with Aicardi-Goutières Syndrome (AGIS). SAMHD 1 appears to restrict infection by depleting the intracellular pool of deoxynucleoside triphosphates, which, in the presence of Vpx, is increased. Presumably, a reduction in intracellular dNTP levels by SAMHD 1 leads to a less permissive environment for reverse transcription of viral cDNA.

The restrictive activities of SAMHD 1 have been conserved throughout the evolutionary history of primates. Degradation of SAMHD 1 by Vpx appears to be species-specific. For example, Vpx from red-capped mangabey SIV (SIV_{rcm}) is unable to degrade human SAMHD 1 but efficiently degrades rhesus SAMHD 1. The cellular differentiation state appears to be essential for the Vpx phenotype and SAMHD 1 restriction activity appears to be specific for differentiated, nondividing cells. All of the biochemical and biologic data obtained to date suggest that SAMHD 1 restricts lentivirus infection only in myeloid cells. Benkirane raised the possibility that SAMHD 1 also restricts viral infection in quiescent CD4+ T cells. SAMHD 1 appears to be expressed efficiently in quiescent CD4+ T cells and does not require activation to increase expression. However, Vpx does not appear to have the capability to degrade SAMHD 1 in quiescent CD4+ T cells. Although SAMHD 1 exhibits properties that would be predicted by the Vpx-associated restriction, it is unlikely to act independently. For example, expression levels of SAMHD 1 do not collaborate with levels of antiviral restriction and expression of SAMHD 1 in T-cell lines such as SUP-T1 does not reconstitute the restriction. Research continues in order to fully understand the biochemical nature of the restriction and cofactors of SAMHD 1 that are necessary for its full biologic activity.

Although a number of studies have demonstrated that SAMHD 1 is active against all primate lentiviruses including HIV-1, HIV-1 does not encode Vpx. Although HIV-1 contains a *vpr* gene, there is no evidence that HIV-1 Vpr is able to neutralize SAMHD 1—a central question is why HIV-1 has not evolved a strategy to do so. Littman and researchers demonstrated that HIV-1 infection of myeloid cells does not normally induce an interferon response.[4] However, if Vpx is introduced in the form of virus-like particles, the infection induces an interferon response. It is tempting to speculate that HIV-1 has deliberately avoided evolving a strategy to neutralize SAMHD 1 to stay below the radar of the interferon response. This suggests a fundamental difference in the biologic properties of HIV-1 and HIV-2/SIV. Given this information, it is therefore puzzling that HIV-1 retains the capability to infect myeloid lineage cells even though it is

unable to restrict SAMHD 1. It is possible that HIV-1 has adapted to undergo reverse transcription in low-dNTP environments and is therefore only partially analogized by SAMHD 1. There is much work to be done in understanding how myeloid lineage cells impact primate lentivirus pathogenesis. Clearly, these viruses have evolved strategies to infect myeloid lineage cells and to evade myeloid-specific restrictions (at least in the case of HIV-1 and SIV). This supports the notion that myeloid lineage cells play a crucial role in the biology of these viruses.

In the same session, Luban outlined the state of research on TRIM5α, as a restriction factor that recognizes HIV-1 capsid (Abstract 65). The existence of this restriction was first suggested by the realization that HIV-1 inefficiently infects monkeys, which correlated with an inability of HIV-1 to replicate within cells of many monkey species. While working in Sodroski's laboratory, Stremlau identified TRIM5α as the factor that restricts HIV-1 infection of monkey cells.[5] Around the same time, Luban's lab identified cyclophilin A as a factor that restricts HIV-1 infection of owl monkey cells.[6] Both proteins recognize viral capsids through a domain that regulates species-specific restriction. Amino acid differences within this domain result in changes in activity such that HIV-1 is recognized by monkey TRIM5α, but not by human TRIM5α. Some structural insight into some of the domains within TRIM5α, such as RING and B-box domains, has been obtained, but there is no structural information on the complete protein. This has hampered attempts to gain detailed insight into how TRIM5α acts on the viral capsids. The most plausible model is that TRIM5α interacts with the capsid to promote premature uncoating. TRIM5α has also been shown to associate with proteasome components, suggesting that another part of the restriction mechanism may involve the proteasome. Ubiquitinylation of any viral component has yet to be observed.

Luban went on to discuss the possibility that TRIM5α, through recognition of the capsid lattice, serves as an innate pattern recognition receptor that alerts the infected cell to the incoming viral particle. In support of this hypothesis, Luban presented data that over-expression of TRIM5α activates innate immune transcription factors such as activating protein 1 (AP-1) and nuclear factor κB (NF-κB), and TRIM5α knock-down inhibits lipopolysaccharide (LPS) signaling in human dendritic cells. Further, challenge of dendritic cells with retroviruses restricted by TRIM5α activated inflammatory cytokines. TRIM5α appears to activate AP-1 and NF-κB through the transforming growth factor β-activated kinase 1 (TAK1) and TAK2/TAK3 complex. This kinase complex has ubiquitin binding components (TAK1-binding protein-3 [TAB3]). Using purified individual components, TRIM5α was shown to synthesize unattached lysine-63–linked ubiquitin chains that are unattached to any substrate. TRIM5α perhaps stimulates these chains to activate TAK1 phosphorylation. Luban went on to demonstrate that HIV-1 capsid lattices stimulate this activity. Collectively, these data indicate that TRIM5α is a pattern recognition receptor and that the retroviral capsid lattice is the pathogen-associated molecular pattern (PAMP).[7] Furthermore, the TAK1 complex is activated by TRIM5α-mediated restriction. Therefore, in addition to the premature uncoating and proteasome mechanisms previously shown in TRIM5α-mediated restriction, an additional aspect to this restriction involves K63-linked ubiquitination, activation of TAK1, and an additional level of restriction. How activation of TAK1 contributes to TRIM5α restriction is under investigation.

A new dimension on the TRIM5α story is the possibility that TRIM5α plays a role in pathogenesis. Monkey TRIM5α does not effectively target SIV capsids. However, there is a growing body of literature to suggest that TRIM5α may modulate control of SIV replication in rhesus monkeys,[8] although this is not seen consistently.[9] There are less solid data regarding the impact of TRIM5α polymorphisms in humans, although some papers suggest that these polymorphisms impact HIV-1 acquisition and disease progression. To this end, Abstract 237 described a G249D polymorphism that is a common variant in Asians and is associated with an increased susceptibility to HIV-1. There are also data to suggest that TRIM5α may play a role in control of HIV-1 infection in individuals who have particular human leukocyte antigen (HLA) genotypes and who mount strong cytoxic T lymphocyte (CTL) responses to *gag*. CTL escape variants acquired mutations that impact viral fitness by increasing susceptibility to TRIM5α restriction.

Viral Reservoirs and Persistence

Much attention has focused on the mechanism with which HIV-1 persists in the face of antiretroviral therapy and this topic received extensive coverage at the conference. A reservoir of quiescent, latently infected CD4 + T cells is considered to be the single biggest obstacle to viral eradication. When HIV-1 is in a latent state, it is not affected by the antiretroviral drugs currently used in the management of HIV-1-infected individuals. A number of groups have been exploiting approaches to reactivate viral latency with the expectation that reactivated virus can be killed by immune surveillance or attacked with retroviral reagents.

Lewin (Abstract 106) reviewed latency and its maintenance, as well as clinical studies aimed at clearing the latent viral reservoirs. She described a clinical study using vorinostat (suberoylanilide hydroxamic acid [SAHA]), an histone deacetylase (HDAC) inhibitor that has been shown to activate HIV from latency in vitro. Vorinostat is licensed for the treatment of cutaneous T-cell lymphoma and is undergoing numerous phase II trials for other malignancies. The toxic effects of vorinostat are well described, at least in short-term studies, but it is unknown whether there is any toxicity associated with long-term use.

In a trial to reactivate latent HIV, 20 patients received 14 days of vorinostat. Blood samples were collected frequently and rectal biopsy was conducted at day 0 and day 14. The major

endpoints were cell-associated viral RNA, as well as other indicators of viral activity, such as cell-associated HIV DNA. The study group was very well-suppressed, with a median CD4+ cell count of 710/µL. Some adverse events were reported in 8 of 9 patients. These events have been previously observed in vorinostat trials but reversed on discontinuation of the drug. In rectal biopsies, there was no evidence of T-cell activation at day 0 and day 14. Viral markers are currently being assessed.

In another trial conducted by Margolis and colleagues (Abstract 157LB), patients received a single dose of vorinostat. The primary endpoints were cell-associated viral RNA and frequency of latently infected T cells. Thirteen patients have been enrolled. Extensive baseline viral characteristics were determined for each patient at enrollment. Quiescent lymphocytes were purified and treated ex vivo with vorinostat to ensure that their cells responded to the treatment. Lymphocytes from each of the 6 enrolled patients showed an increase in cell-associated HIV-1 RNA after treatment with vorinostat ex vivo. Following administration of vorinostat, there was a similar induction of cell-associated viral RNA in patients following a single 400-mg dose of vorinostat. There was no significant change in the level of single-copy viral RNA in all patients. This study provides proof-of-concept that a single dose of vorinostat induces expression of full-length viral RNA within resting CD4+ T cells and provides a framework with which to establish an optimal dosing schedule for the drug.

An important consideration in developing strategies to eradiate the latent viral reservoir by reactivation is the expectation that viral cytopathic effects or immune surveillance would accelerate destruction of the reactivated cell. Abstract 153 examined the ability of CD8+ cells to kill latently infected resting T cells that were treated with vorinostat in vitro. CD4+ cells were obtained from patients on suppressive antiretroviral therapy and were used to generate latent infection in vitro. Cells were then reactivated with vorinostat—of the 1% to 3% of cells in latent infection, the majority were reactivated with treatment. Autologous CD8+ cells were then obtained from the same patients and cocultured with SAHA-reactivated cells at a 1-to-1 ratio. CD8+ cells from an elite controller with a high level of cytotoxic T-cell activity efficiently killed HIV-1-infected cells over an 8-day interval. However, CD8+ T cells from patients receiving antiretroviral therapy did not effectively kill latently infected T cells after virus reactivation. Furthermore, there was no obvious cytopathic effect in these cultures. Stimulation of patient CD8+ cells with HIV *gag* peptides enhanced the CTL responses and led to killing of vorinostat-treated cells. It should be noted that latently infected cells were transduced with Bcl-2 in order to maintain their viability, but this could also have affected their susceptibility to cytopathicity and CTL lysis. Nevertheless, Bcl-2 transduced cells died when virus was reactivated by CD3/CD28 co-stimulation. These sobering data suggest that reactivation of viral latency in vivo will be insufficient to accelerate the death of the reactivated cell and will require additional measures to boost cytotoxic T-cell responses in patients undergoing purging protocols to eliminate the latent reservoir.

Despite these apparent setbacks, the field of research in viral reservoirs is engendered with a sense of purpose in pursuing strategies that will achieve viral eradication. Ultimate success will depend on a complete understanding of the nature of the viral reservoirs that persist in the face of antiretroviral therapy, and that understanding will inform the most effective strategies to eliminate those reservoirs.

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A list of all cited abstracts appears on pages 87-93.

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