Factors Influencing Virus–Host Cell Interplay

Mario Stevenson, PhD

The Conference on Retroviruses and Opportunistic Infections (CROI) provides an annual international forum for basic scientists and clinical and global health researchers to present and become current on the most recent advances in the field of HIV and AIDS research. The 18th conference contained a number of strong basic science sessions. HIV-1 infection of the cell is opposed by cellular factors that attack the viral replication cycle at various points. However, the virus has evolved defenses against these innate cellular antiviral proteins. CROI continues to be a strong forum for presentation of the most recent developments in this area of research. In addition, there were numerous presentations on cellular factors that regulate virus–host cell interplay as well as on research that is providing detailed insight into the mechanism of action of integrase inhibitors. Some presentations focused on approaches to studying and intervening with viral latency, particularly in primary cell models. Research on the use of zinc-finger nucleases to knock out CC chemokine receptor 5 expression in CD34+ stem cells also received a lot of interest. The hope is that these strategies will provide new therapeutic approaches to generate resistance to HIV-1 infection.

In the Bernard Fields Lecture, Cullen discussed the role of a novel class of small regulatory RNA molecules, called microRNAs (miRNAs), in the replication of viruses, in particular, herpes viruses (Abstract 17). MiRNAs bind to messenger RNA (mRNA) molecules that have partially or fully complementary sequences and as a result are able to impair mRNA translation and reduce mRNA stability. Cullen described miRNAs that play a central role in the regulation of herpes virus latency as well as virus-encoded miRNAs that regulate cellular functions important for virus survival in the host. The search for miRNAs that regulate HIV-1 replication is ongoing, and whether there are microRNAs encoded by HIV-1 is still a matter of debate. Cullen discussed the role of virus-encoded miRNAs in the maintenance of Epstein-Barr virus latency. He described how miRNAs participate not only in the maintenance of latency, but also in maintenance of B-cell transformation that is important to sustain the viral reservoir.

Viral Replication and Cell Cycle

Infection of a cell is initiated by interactions of the viral envelope glycoprotein with receptor and coreceptor molecules on the cell surface. Exposure of fusogenic domains on the viral envelope leads to fusion between viral and cellular membranes; this fusion allows the viral core, which contains the viral nucleic acid, to be inserted into the cytoplasm of the target cell. In quiescent CD4+ T cells, infection events leading up to establishment of a complementary DNA (cDNA) are inefficient. However, latent infection is established in quiescent T cells, and it is unclear how latency is initiated. One possibility is that some infection events in cycling CD4+ cells result in latent infection if the cell rapidly returns to a state of quiescence after establishment of the integrated provirus. An alternative possibility is that binding of the virus to a quiescent cell may engender a signal that would increase the permissivity of the cell to infection, thereby facilitating the establishment of a latent infection.

Abstract 174 presented evidence that quiescent T cells can be infected through an endosomal route. The authors produced HIV-1 variants that were pseudotyped with an R5-tropic HIV-1 envelope and with the fusogenic G glycoprotein of vesicular stomatitis virus (VSV-G). They demonstrated that both CD4+-dependent binding and subsequent VSV-G fusion were necessary for infection of naive CD4+ T cells. This study could be important because it would provide an approach for the establishment of latent infection in vitro, thereby allowing detailed investigation of the mechanisms regulating latency in a physiologically relevant model.
Virologic Synapse: Sensitivity to Neutralization

In lymphoid tissue, where there is a high density of substrate CD4+ T cells, viral spread may occur not only as free viral particles but also directly between cells through cell-to-cell contact. HIV-1 has been reported to form virologic synapses (VS) between the HIV-1-infected cell and the target cell’s interface. Formation of VS involves interaction between the viral envelope and CD4+ cell-coreceptor complexes and further requires cytoskeletal rearrangements and stabilization of infected and target-cell membranes by adhesion molecules. The nature of the VS has prompted speculation that this mode of viral transfer may afford the virus some degree of protection from neutralizing antibodies or from inhibitors that prevent interactions with receptor and coreceptor molecules.

Research presented in Abstract 181 provided evidence that the VS may provide an infection route that is less sensitive to some broadly neutralizing antibodies. The investigators observed that although most neutralizing antibodies blocked both cell-free and cell-associated HIV-1 infection, a 17b antibody that is reactive against a CD4-induced binding site of envelope glycoprotein as well as patient serum both inhibited cell-free infection more effectively than they inhibited VS-mediated infection. Deletion of the gp41CT enhanced sensitivity to 17b after VS-mediated infection.

Abstract 182 presented evidence that 2- to 3-fold higher concentrations of entry or fusion inhibitors including maraviroc, enfuvirtide, and the investigational drug AMD 3100 were required to disrupt transinfection between mature dendritic cells and CD4+ T cells than were required for cell-free virus infection of CD4+ T cells.

Abstract 89 presented evidence for cellular factors that regulate envelope incorporation into the virion. During assembly of the HIV-1 particle at the plasma membrane, the viral envelope glycoprotein is incorporated into the budding virus particle. The cellular protein Rab11a plays a central role in the recycling of cellular glycoproteins to the plasma membrane. The authors demonstrated that a dominant negative mutant of Rab11a did not affect envelope incorporation, but expression of a constitutively active form of Rab11a appeared to direct the envelope glycoprotein to a cellular location for degradation, thus dramatically decreasing envelope incorporation into released virions. Therefore, the Rab11a family of interacting proteins appears to be a key mediator of viral envelope glycoprotein trafficking.

HIV/Flavivirus GB Virus C Coinfection

The regulation of cellular activation state and CC chemokine receptor 5 (CCR5) down-regulation were reported to provide mechanisms for limiting HIV-1 target-cell availability in individuals coinfected with the flavivirus GB virus C (GBV-C). GBV-C infects approximately 30% of individuals with HIV-1, and coinfected individuals have longer average survival times than HIV-1-infected individuals without GBV-C. The mechanisms underlying the GBV-C benefits are not well understood.

Abstract 26 examined the frequencies of CCR5+ CD4+ T cells in GBV-C-seropositive and -seronegative HIV-1-infected individuals. The investigators determined that levels of the cytokine RANTES (regulated on activation normal T-cell expressed and secreted), a ligand for CCR5, were higher in GBV-C-seropositive individuals than in GBV-C-seronegative subjects. The plasma level of RANTES was inversely correlated with the frequency of CCR5+ CD4+ memory T cells, which itself was related to the median fluorescence intensity of CCR5. Therefore, one of the mechanisms underlying the survival benefits conferred by GBV-C infection might involve higher plasma levels of CCR5-binding chemokines, subsequent down-regulation of CCR5 expression, and a reduction in target-cell availability.

Abstract 27 presented additional evidence for decreases in CD4+ and CD8+ cell activation and proliferation in GBV-C/HIV-1–coinfected individuals compared with HIV-1-infected individuals without GBV-C. CD4+ cell count and percentage were statistically significantly higher in GBV-C-seropositive individuals than in GBV-C-uninfected subjects.

However, in contrast to results from Abstract 26, there were no differences in CCR5 or CXC chemokine receptor 4 (CXCR4) expression on CD4+ cells between GBV-C-seropositive and -negative subjects. In individuals who had detectable HIV-1 viremia, CD4+ cell proliferation was lower in GBV-C-seropositive subjects than in GBV-C-uninfected subjects, and activation levels of CD4+ cells and CD8+ cells were also lower in this group. Therefore, GBV-C viremia appears to be associated with decreased CD4+ T-cell proliferation and activation, which may contribute to improved survival in HIV-1 subjects coinfected with GBV-C.

Studies on Viral Uncoating During Infection

After fusion of HIV-1 with the host cell surface, the viral capsid core enters the cytoplasm. The viral capsid deassembles to release viral nucleic acids into the cytoplasm to be reverse-transcribed. How the reverse transcription and uncoating processes are coordinated during infection remains poorly understood. Abstract 90 presented evidence that drugs that inhibit reverse transcription delayed the uncoating process. The study used a fluorescence-based uncoating assay as well as an owl monkey kidney cell line assay that manifests TRIM-CypA-mediated restriction.

Data from both assays indicated that uncoating is initiated within an hour of viral fusion. Inhibition of reverse transcription using nevirapine delayed uncoating from approximately 40 minutes to 2 hours. Furthermore, analysis of reverse transcription products in owl monkey kidney cells indicated that appearance of early reverse transcription products coincided with the initiation of uncoating. This suggests that reverse transcription facilitates, but is not required for, HIV-1 uncoating in infected cells.
Viral Integration

In a plenary presentation, Cherepanov presented structural insights into the process of retroviral integration and the mechanism of action of strand-transfer inhibitors (Abstract 75). After reverse transcription, the viral integrase enzyme binds to the viral DNA ends to form a stable nucleoprotein complex, the intasome. In the nucleus, the intasome interacts with target-cell DNA and catalyzes the joining of viral DNA with cellular DNA. Using the prototype foamy virus (PFV) intasome as a model system, Cherepanov presented the crystal structure of the PFV intasome, which comprises an integrase tetramer tightly associated with a pair of viral DNA ends. Integration inhibitors, including raltegravir, elvitegravir, and related strand-transfer inhibitors, were found to bind within the active site of the PFV intasome to dislocate the reactor viral DNA. Furthermore, because of strong sequence conservation within the active sites of PFV and HIV-1 integrase, the mechanism by which raltegravir resistance mutations impact antiviral activity could be visualized within the PFV intasome model. The availability of crystal structures for the retroviral intasome is an important step in the development of next-generation integrase inhibitors.

Integration of viral DNA into host chromatin is not a random process but appears to occur preferentially in transcription units. Lens epithelium-derived growth factor (LEDGF) is a cellular cofactor of HIV-1 integrase that promotes viral integration into gene-rich regions of host chromatin. Abstract 191 examined the ability of HIV-1 to replicate in the absence of LEDGF in a human LEDGF knock-out cell line. In the absence of LEDGF, integration did not occur in transcription units but instead, showed a preference for the genomic regions termed CpG islands. Although spreading HIV-1 replication was delayed in the knock-out cell line, residual replication was observed. Nevertheless, the authors demonstrated that this residual replication was still sensitive to the investigational compound termed LEDGIN, which interrupts the interaction between HIV-1 integrase and LEDGF. These data reinforce the critical role of LEDGF as a cofactor in HIV-1 integration. The mechanism by which HIV-1 is able to integrate in the absence of LEDGF is under investigation.

Mechanisms of Latency

The site of integration has also been proposed as an important component of the mechanism by which HIV-1 latency is regulated. Both HIV-1 integration and latent proviruses appear to occur in actively expressed host genes. As a consequence, transcriptional interference between the HIV-1 long-terminal repeat (LTR) and the juxtaposed cellular transcription unit has been proposed in epigenetic regulation of HIV-1 latency. Further understanding of the mechanisms regulating HIV-1 latency, Abstract 197 described the development of a dual promoter system that examined the interaction between the HIV-1 LTR and upstream promoters. The authors demonstrated that the maintenance of latent HIV-1 infection depends upon the level of expression of the gene proximal to the provirus. This provides a model for how latent infection can be established in actively expressed cellular genes.

Equally important to an understanding of the mechanisms by which latency is established is an understanding of the processes that can trigger reactivation from latency. Because the latent reservoir is considered the most important obstacle to viral eradication, many investigators have turned their attention to strategies that reactivate latency to reduce the size of the latent reservoir. In Abstract 198, Wightman and colleagues described the development of a primary resting-T-cell model of HIV latency. In this model, resting CD4+ T cells are incubated with the CC chemokine receptor 7 (CCR7) ligand CCL19. The authors have previously demonstrated that treatment of resting CD4+ T cells with certain chemokines such as CCL19 is sufficient to render them permissive to HIV-1 infection.

They used this model to examine the potency and toxicity of histone deacetylase (HDAC) inhibitors and other immune activators in reactivating HIV-1 latency. The authors observed statistically significant variation in the potency and toxicity of a variety of HDAC inhibitors, including the lymphoma drug vorinostat and the investigational cancer drugs pamabino- stat and etinostat as well as cytokines including interleukin 7 (IL-7), tumor necrosis factor alpha (TNF-α), and prostratin. HDAC inhibitors showed varying degrees of activity in the primary model of HIV-1 latency. However, toxicity in peripheral blood mononuclear cells was also observed with concentrations close to those required to reactivate HIV-1 latency. Vorinostat exhibited the lowest toxicity and highest potency in the primary T-cell model of HIV-1 latency, underscoring a potential role for this drug in clinical trials to eliminate the resting cell reservoir.

Cellular Restrictions

The area of cellular restrictions continued to draw strong interest at the conference. As a virus with a limited genetic repertoire, HIV-1 commandeers cellular factors at various stages in its replication cycle. The seminal discovery, by Malim’s research group, of the cellular restriction APOBEC 3G revealed the existence of cellular factors that oppose viral infection. Since that discovery, additional cellular factors that antagonize viral replication have been identified. For example, TRIM5α exerts a species-specific effect on viral uncoating, and tetherin/BST2 interferes with disassociation of viral proteins from the cell surface. The existence of these potent antiviral restrictions has forced primate lentiviruses to adopt evasion strategies, and almost all of the viral counterdefenses are directed by the viral accessory proteins. The Vif protein antagonizes APOPEC 3G by promoting its premature proteasomal destruction, and the protein Vpu antagonizes tetherin/BST2 by directing it away from sites of virus assembly. Because Vpu is not encoded by the majority of SIV variants or by HIV-2, an important question is how these viruses evade the antiviral action...
of tetherin/BST2. Studies by Evans previously established that SIV Nef proteins antagonize tetherin/BST2. Abstract 85 presented further insight into the mechanism by which SIV Nef antagonizes tetherin/BST2. Nef proteins have previously been demonstrated to bind adaptor protein 2 (AP-2). SIV Nef mutants incapable of binding AP-2 were unable to antagonize tetherin, and down-regulation of tetherin/BST2 from the cell surface was observed with wild-type Nef but not with AP-2-binding-site mutants. Therefore, SIV Nef proteins antagonize tetherin/BST2 in an AP-2-dependent mechanism that allows displacement of tetherin/BST2 from the site of virus assembly or down-regulation of tetherin/BST2 from the cell surface.

Recent studies from our research group have presented evidence that primate lentiviral Vpx proteins enhance infection of myeloid cells by antagonizing a cellular restriction. Although the identity of the restriction as well as its viral target are yet to be revealed, the restriction appears to be a major barrier to infection of myeloid cells. Our published studies have demonstrated that although HIV-1 Vpr is unable to neutralize the myeloid cell restriction, HIV-1 is sensitive to restriction, and packaging of Vpx within HIV-1 substantially increases its infectivity for macrophages.

In Abstract 87, Sunseri and colleagues presented evidence that increasing HIV-1 infectivity for macrophages and dendritic cells after Vpx packaging within HIV-1 virions led to an enhanced innate immune response. Vpr and Vpx proteins are packaged into virions through association with the p6 domain of Gag. The authors constructed a chimeric HIV-1 Gag containing the SIV p6 domain, and this allowed packaging of Vpx into HIV-1 virions. HIV-1 that was engineered to package Vpx was more infectious in both macrophages and dendritic cells and induced a strong type 1 interferon response. It is intriguing to speculate that HIV-1 has evolved to lack a strategy to neutralize a myeloid cell restriction in order to avoid activating an innate immune response. Therefore, it would be important to determine whether the ability of HIV-1 to remain sensitive to the myeloid cell restriction impacts its biology within the host.

Abstract 28LB presented evidence that some form of antiviral restriction may be playing a role in viral control within elite controllers. Research from Crumpacker and colleagues demonstrated that the cyclin-dependent kinase inhibitor p21 restricted HIV-1 infection of primary hematopoietic cells. In Abstract 28LB, Huang and colleagues examined p21 expression in CD4+ T cells from elite controllers and HIV-1 progressors. Expression of p21 was statistically significantly higher in CD4+ T cells from elite controllers, and silencing of p21 by RNA interference increased HIV-1 replication. This group demonstrated that p21 interacted with the cyclin-dependent kinase 9 (CDK9), which is essential for HIV transcriptional elongation. The authors proposed that p21 acts as an inhibitor of CDK9-mediated transcriptional elongation of HIV-1 in elite controllers.

Pathogenesis

Several clear mechanisms have emerged to explain the processes of lentiviral pathogenesis. In pathogenic primate lentiviral infection, viral replication depletes Tc17 cells that are necessary to maintain the integrity of the gut mucosa. A subsequent loss in gut mucosal integrity leads to translocation of bacterial products that drive cell activation, including lipopolysaccharide (LPS). This increases the pool of cells permissive to viral infection and erodes the architecture of the lymphoid tissue. As originally hypothesized by Giorgi and colleagues, immune activation (as measured by frequency of activated CD8+ T cells) correlates with plasma viral load and is a strong predictor of disease progression. Immune activation persists in HIV-1-infected individuals, is not normalized by antiretroviral suppression, and undermines CD4+ cell count gains during treatment.

In one study, no correlation between bacterial translocation and immune activation and low-level viremia was observed in HIV-1-infected individuals who exhibited poor CD4+ T-cell recovery during suppressive antiretroviral therapy (Abstract 304). The researchers examined the association between CD4+ T-cell gains and T-cell activation (CD38+HLADR+CD8 cells), markers of microbial translocation (LPS and 16S ribosomal DNA), as well as monocyte activation (soluble CD14). In 71 participants, no markers of bacterial translocation were associated with CD4+ T-cell counts. Nor was there a correlation of CD4+ T-cell count or immune activation with low-level plasma HIV RNA. There was an association of CD8+ lymphocyte activation with lower CD4+ cell count. Therefore, additional studies are required to identify biological correlates of a nontherapy response in patients receiving suppressive therapy.

Strategies for Viral Eradication

There has been a single documented case of HIV-1 eradication: the “Berlin patient,” an HIV-1-infected individual with leukemia. He received chemotherapy followed by transplanted hematopoietic stem cells from a CCR5-negative donor. This case has provided proof-of-concept for strategies that engineer stem cells to become CCR5-negative. Abstracts 164 and 165 discussed the potential use of zinc-finger nucleases to edit the CCR5 gene in stem cells. In Abstract 164, Cannon and colleagues discussed the use of zinc-finger nucleases to knock out the CCR5 gene in human CD34+ stem cells. Those stem cells were then transplanted into immune-deficient mice. Infection of these mice with R5-tropic strains of HIV-1 led to the preferential survival of CCR5-negative T cells, substantial declines in HIV-1 viremia, and eventual restoration of normal levels of CD4+ T cells in lymphoid tissues of the mouse.

Abstract 165 discussed the application of this strategy to humans in ongoing phase I clinical trials involving a group of patients for whom 2 or more antiretroviral regimens have failed and who remain viremic and a second
group of patients who are doing well on antiretroviral therapy. In this study, the CCR5 locus is disrupted with zinc-finger nucleases, and then the CCR5-deficient T cells are transplanted into the patients.

Although these are exciting studies, an important question arises as to whether the preexisting viral reservoirs will persist in individuals who receive transplanted CCR5-negative cells. The success of the Berlin patient likely hinged on the fact that the existing reservoir was depleted through chemotherapy. However, in allogeneic stem cell transplantation, for which chemotherapy is not necessary, the preexisting latent reservoir is likely to persist. Therefore, at best, CCR5 removal through allogeneic stem cell transplantation may offer a means for viral control but may not achieve the goal of viral eradication.

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A list of all cited abstracts appears on pages 99–106.

References