

Review

CROI 2015: Basic Science Review

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The 2015 Conference on Retroviruses and Opportunistic Infections (CROI) represents a forum that encompasses all facets of research on HIV/AIDS and its complications. CROI is a valuable venue for scientific and public health researchers, clinicians, policy makers, and community representatives to be updated on the latest advances in their specific areas of interest and beyond. CROI 2015 continued to surprise. New insights into the viral reservoirs that persist in the face of antiretroviral therapy were prominently featured, as were therapeutic approaches aimed at curtailing and eliminating persistent viral reservoirs in HIV-infected individuals. Basic science is providing surrogates that could be valuable in how viral reservoirs are measured and, ultimately, in how to gauge if they are being effectively eliminated.

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Viral Reservoir Studies

Development of a strategy to cure HIV-1 infection and development of an efficacious vaccine are the most challenging undertakings in HIV/AIDS research, as was discussed at the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 22 to 26. Although antiretroviral therapy has profoundly improved, the prognosis for individuals living with HIV/AIDS—the prospect of lifelong antiretroviral therapy—is not ideal for a number of reasons. Emergence of resistant viruses limits the effectiveness of antiretroviral drugs, ongoing immune inflammation leads to collateral damage even in virally suppressed individuals, and maintaining adherence will continue to be challenging for HIV-1–infected individuals on long-term therapy. Adherence may be affected by drug toxicity, cost of medications, mental health issues, and substance use. For this reason, a coordinated effort to develop therapeutic approaches that can lead to a viral cure is needed.

HIV-1 has adopted strategies with which to achieve lifelong persistence in the human host. This is perhaps

best illustrated in a recent study by Henrich and colleagues in which 2 HIV-1–infected individuals underwent allogeneic hematopoietic stem cell transplantation (HSCT) for hematologic cancers.¹ In contrast to those in the Berlin patient,² donor cells in the 2 individuals in this study expressed the full complement of viral coreceptor molecules. The objective was to determine whether a component of allogeneic HSCT could promote a sustained remission from antiretroviral therapy. HIV-1 DNA was undetectable in peripheral blood and rectal mucosa following allogeneic HSCT and provided justification for interrupting antiretroviral treatment. Plasma HIV-1 RNA and cell-associated HIV-1 DNA were undetectable in these 2 individuals until 12 weeks and 32 weeks, respectively, after cessation of antiretroviral therapy. Therefore, although allogeneic HSCT led to an extended period of antiretroviral therapy–free remission in 1 individual, the virus still rebounded, indicating the presence of long-lived tissue reservoirs of HIV-1. Despite this sobering result, there are renewed efforts to chart the viral reservoirs that sustain HIV-1 in the face of antiretroviral therapy.

Mechanisms of Reservoir Persistence

Most of the attention with regard to viral reservoir persistence has focused on the reservoir of resting memory CD4+ T cells harboring latent HIV-1. The true longevity of latently infected CD4+ T cells is unknown, but given the immunologic role of memory CD4+ T cells, to recognize antigens in adulthood that were first encountered in childhood, it is likely to extend over years. The true longevity of viral reservoirs is further complicated by the fact that some memory CD4+ T cells undergo homeostatic proliferation, during which integrated latent proviruses could be duplicated during formation of daughter cells at mitosis, as originally demonstrated by Chomont and colleagues.³ Although it is possible that latent and replication-competent proviruses can be duplicated through the process of homeostatic proliferation, the majority of proviruses amplified through this process would be expected to be defective, non-replication-competent proviruses. A number of studies have revealed that the vast majority of proviruses of HIV-1–infected individuals harbors deletions and hyper mutations that render the genome nonfunctional. Therefore, the extent to which homeostatic proliferation maintains a reservoir of replication-competent proviruses is unknown.

As featured in several presentations at last year's CROI,⁴ there is clear evidence for duplication of proviruses at mitosis, as evidenced by the presence of identical clonal proviruses in peripheral CD4+ T cells from HIV-1–infected individuals taking suppressive antiretroviral therapy. In his plenary presentation at CROI 2015 (session PL-2),

Hughes summarized a comprehensive analysis of HIV integration sites in peripheral blood mononuclear cells (PBMCs) from HIV-infected individuals taking suppressive antiretroviral therapy (Abstract 21). Of 2500 integration sites, 40% were in clonally expanded cells. Indeed, in 1 HIV-infected individual, more than 50% of the proviruses had emerged from a single clone. Proviruses with identical integration sites can only be the result of mitotic duplication of proviruses during clonal cell expansion. Further, some integration sites could drive cell proliferation if those sites result in dysregulation of a gene that is involved in cell proliferation. In such a case, an increased frequency of integration sites within specific genes and an increased frequency of identical integration sites within that gene would be seen. Numerous independent integrations in the same integration site were observed in 2 introns of the MKL/myocardin-like 2 (MKL2) and BTB and CNC homology 1, basic leucine zipper transcription factor 2 (BACH2) genes, which are involved in cell growth regulation. Some integration sites were identical, further indicating clonal expansion of cells harboring proviruses at those integration sites. Given the potential for insertional activation of cell growth-regulating genes, it is surprising that cancers are not reported at a higher frequency in HIV-1-infected individuals. Although integration near growth regulating genes provides an opportunity for maintenance of proviruses, a central question is whether this provides a mechanism for the maintenance of functional proviruses in the viral reservoir.

Cell proliferation, as driven by viral integration site, promotes proviral duplication.

Studies of Activity of the Viral Reservoir

A major challenge is identifying latently infected cells in HIV-1-infected individuals. Currently, latency is considered to comprise resting memory CD4+ T cells harboring transcriptionally

dormant proviruses. As such, other than by the presence of viral DNA, a latently infected cell would be indistinguishable from an uninfected cell. Several presentations focused on examining the biologic significance of cell-associated viral RNA that can be detected in individuals taking suppressive antiretroviral therapy. Simonetti and colleagues (Abstract 105) previously identified a clonally expanded population of HIV-1-infected cells that was responsible for persistent viremia in a patient with metastatic squamous cell carcinoma. Antemortem and postmortem analyses were conducted to determine the tissue origin of this clonal variant. One provirus from a highly amplified clone, which produced the majority of viral RNA in plasma, was found to be enriched in tumor tissues. Further, this provirus yielded virus in cultures *ex vivo* when cocultured with CD4+ T cells. Therefore, biologically competent proviruses can be amplified through clonal expansion.

Similarly, Wiegand and colleagues (Abstract 106) conducted genetic analysis of cell-associated RNA in aviremic individuals to gauge their relationship to proviral populations and persistent viremia. Two aviremic and 1 viremic individuals were assessed. The frequency of G-to-A hyper mutations was detected at similar levels to their proviral DNA populations, and RNA was also found to match a clonally expanded population in 1 individual. As yet, it is unclear whether cell-associated RNA is a result of spontaneous reactivation of latently infected cells or whether the viral reservoir constitutes cells undergoing continuous low-level viral transcription. Further, whether the cells can be a source of virus if antiretroviral therapy is interrupted remains to be determined.

In an attempt to gauge the biologic significance of cell-associated viral RNA, Etemad and colleagues (Abstract 110LB) performed a retrospective analysis of HIV-1-infected individuals from 5 AIDS Clinical Trials Group (ACTG) studies who were virologically suppressed on antiretroviral therapy and who underwent analytic

treatment interruption (ATI). The timing of virus rebound was based on a confirmed HIV-1 plasma RNA level of greater than 200 copies/mL or a single HIV-1 RNA level of greater than 1000 copies/mL. Individuals initiating antiretroviral therapy during early or acute HIV-1 infection had lower levels of pre-ATI viral RNA than those treated during chronic infection. Levels of cell-associated RNA were associated with time to viral rebound after interrupting antiretroviral therapy. It remains to be determined whether time to rebound following ATI can serve as a surrogate for the size of the viral reservoir that persists in the face of antiretroviral therapy. According to current models, viral recrudescence occurs following stochastic reactivation of a small number of founder, latent viruses. Because of the stochastic nature of the reactivation, time to reinitiation of detectable viremia might be highly variable and, as such, not a reliable measure of the reservoir. However, a recently published study from Rothenberger and colleagues indicates that viral recrudescence is fueled by the simultaneous reactivation of founders in lymphoid tissue.⁵ This suggests the viral reservoir is larger and more active than previously suspected.

Bull and colleagues (Abstract 107) presented studies aimed at identifying the source of low-level viremia that persists in individuals taking suppressive antiretroviral therapy—evidenced by HIV-1 RNA level between 40 copies/mL and 500 copies/mL after 1 year of suppressive antiretroviral therapy. Sequences in the viral envelope region (C2-V5) were correlated with the integration sites using an integration site looping assay.⁶ Analysis of integration sites was able to determine whether proviruses were present in proliferating cells and if they had been duplicated through cell division. Proviruses with identical integration sites were observed in 6 of 8 subjects who exhibited low-level viremia at repeated study visits. Two of the 6 subjects exhibited proliferating clones that had envelope sequences identical to those in low-level viremia, and 3 of the 6 subjects had sequences of

low-level viremia that were not linked to proliferating PBMC sequences and had evidence of ongoing viral evolution, suggesting that viremia was the result of residual viral replication. This study further underscores observations that duplication of proviruses during cellular proliferation can maintain a population of functional proviruses that can also contribute to low-level viremia that persists in individuals taking suppressive antiretroviral therapy. Collectively, these studies suggest that in patients taking suppressive antiretroviral therapy, HIV-1–infected cells and –uninfected cells may be distinguishable by more than the presence of proviral DNA. If a component of the reservoir is transcriptionally active and some of those transcripts are translated, then it could be predicted that that component is amenable to clearance by immune-based strategies such as therapeutic vaccination. More studies are required to determine the longevity of the transcriptionally active viral reservoir and its modulation by host immune responses.

An active viral reservoir may persist in individuals taking suppressive antiretroviral therapy.

Immunologic and Virologic Surrogates of Posttreatment Control

Hurst and colleagues (Abstract 111LB) retrospectively analyzed samples from SPARTAC (Short Pulse Antiretroviral Therapy at HIV Seroconversion), a randomized study of primary HIV infection that incorporated a treatment interruption after 48 weeks of antiretroviral therapy. A battery of immunologic and virologic endpoints was assessed to determine if any of those markers could predict the extent of posttreatment control after treatment interruption. T-cell exhaustion markers, such as T-cell immunoglobulin domain– and mucin domain–containing molecule-3 (TIM-3) in CD8+ T cells and programmed cell death 1 (PD-1) TIM-3 and lymphocyte-activation gene 3 (LAG-3)

in CD4+ T cells, were associated with time to rebound when measured pre-therapy. However, other than total viral DNA, there did not appear to be any viral markers associated with time to rebound when measured at baseline or at treatment interruption.

Non-CD4+ T-Cell Reservoirs

A long-standing debate in the field is the contribution of cells other than CD4+ T lymphocytes to viral persistence, particularly in the face of suppressive antiretroviral therapy. Although there is ample experimental evidence that tissue macrophages support viral replication in the simian immunodeficiency virus (SIV)-macaque model and in viremic HIV-1–infected individuals, there is less evidence supporting a role for myeloid cells in aviremic individuals. One of the challenges to assessing the contribution of myeloid cells to viral persistence is the difficulty in sampling tissue macrophage populations of sufficient purity and quantity to determine their infection status. In addition, macrophages are a heterogeneous population of cells residing in various locations, including the liver, lung, bone marrow, spleen, lymph nodes, and gut. Therefore, infection status in 1 population may not reflect what is happening in other populations. Kandathil and colleagues (Abstract 380) examined whether liver macrophages, also known as Kupffer cells, were an HIV-1 reservoir in individuals taking suppressive antiretroviral therapy. Purified liver macrophages from 3 human donors were infected with an R5-tropic GFP reporter virus and culture supernatants assessed for the presence of viral RNA. In addition, liver macrophages were purified from tissue explants taken from HIV-1–infected individuals who were viremic (n = 1) or aviremic (n = 2). Liver macrophages infected in vitro supported the production of infectious virus for up to 6 months. Liver macrophages from both individuals taking suppressive antiretroviral therapy released infectious variants that could transmit to reporter cells, as evidenced by the presence of proviral DNA in those

reporter cells. Collectively, these data suggest low-level infection of liver macrophages in these individuals taking suppressive antiretroviral therapy, and additional studies are required to determine how generalizable this observation is. In addition, liver macrophages are difficult to access, therefore it will be important to evaluate whether more accessible tissue compartments, such as in the lungs and lymph nodes, harbor infected macrophages in individuals taking suppressive antiretroviral therapy.

Viral Tropism Studies

Another approach to assessing the contribution of macrophages to viral persistence is to examine the tropism of variants in plasma of HIV-1–infected individuals and those who undergo treatment interruption. Bednar and colleagues (Abstract 221) looked for evidence of macrophage tropism in viruses in the blood of HIV-1–infected individuals with late-stage infection. A primary determinant of macrophage tropism is the ability to use low levels of CD4. Previous studies from this group have demonstrated that macrophage-tropic viruses, gauged by the ability to use low levels of CD4 on the Affinofile cell line, are infrequent. The study determined whether macrophage-tropic viruses might be present in blood. Analysis of 18 subtype B and 20 subtype C, late-stage, HIV-1–infected individuals did not reveal examples of macrophage-tropic virus. However, some individuals harbored viruses that were more capable of infecting cells with low CD4 levels than typical R5 T-cell-tropic virus. That intermediate CD4 usage phenotype was previously seen in the cerebrospinal fluid (CSF) and in the genital tract. Further, this new group of intermediate viruses showed increased sensitivity to soluble CD4 that approximates what is seen with macrophage-tropic viruses. The investigators proposed that the appearance of intermediate phenotypes in the blood suggests some evolution toward macrophage tropism in compartments that are the origin of viruses in the blood in late-stage disease.

Rebounding viruses may originate from CSF in effectively virologically suppressed individuals.

In an extension of this analysis, the same investigators examined the CD4 usage of viruses that rebounded after treatment interruption (Abstract 112LB). Single genome amplification of the viral envelope gene was conducted to determine sequence diversity in the rebounding virus population. In addition, viral envelopes were cloned to examine tropism on the basis of CD4 usage. Phylogenetic analysis of viral envelope genes demonstrated that recrudescence of the viral population was from a small number of founder variants. Rebounding variants required high levels of CD4 for infection, and there was no evidence of macrophage-tropic virus. This analysis detected no myeloid cell source during viral recrudescence when antiretroviral therapy is interrupted. However, in this study, viruses were being sampled in blood, which does not exclude the possibility that viruses originating from tissue macrophages remain highly localized and tissue bound and do not enter the blood following treatment interruption. This was suggested in a presentation by Gianella and colleagues (Abstract 58). In that study, paired blood and CSF samples were collected from 14 chronically HIV-1-infected individuals taking suppressive antiretroviral therapy. At the earliest 2 time points after viral rebound, viral envelope and reverse transcriptase regions were amplified from cell-free HIV-1 RNA in blood and CSF. Ten of 14 participants demonstrated compartmentalization in viral sequences between blood and CSF in at least 1 gene. Seven participants exhibited very rapid recrudescence of viremia in CSF, suggesting that rebound originated within the central nervous system rather than viruses migrating from the periphery. This suggests that HIV reservoirs in the central nervous system contribute to viral rebound and that in the tissues, rebounding viruses may remain compartmentalized, thus escaping analysis when plasma is sampled.

Strategies for Reservoir Elimination

A number of presentations outlined ongoing efforts to eliminate long-lived reservoirs in individuals taking suppressive therapy. Because the reservoir of latently HIV-1-infected memory CD4+ T cells is considered the single biggest obstacle to viral eradication, investigators have been exploring “shock-and-kill” approaches designed to reactivate the virus from latency and render it susceptible to host immune responses or clearance by viral cytopathic effects on the host cell. Latency is considered to be primarily regulated at the level of transcription where proviruses in highly condensed regions of chromatin are not accessed by transcription factors that are necessary for efficient viral gene expression. Therefore, chromatin-modifying agents (eg, histone deacetylase [HDAC] inhibitors) relax chromatin and allow transcription factors to interact with proviruses and activate viral gene expression. Most of the focus to date has been on the impact of latency-reactivating agents on viral RNA levels. However, it will be necessary to reactive gene expression to the level at which viral proteins are being made if viral cytopathicity or host immune-mediated clearance are to take effect.

Several presentations examined the impact of Toll-like receptor 7 (TLR7) agonists in elimination of latently infected cells. Sloan and colleagues (Abstract 417) demonstrated that the investigational TLR7 agonist GS-9620 activated the virus *ex vivo* in PBMCs of HIV-1-infected individuals taking suppressive antiretroviral therapy. GS-9620 is a selective TLR7 agonist that is currently being evaluated in patients with chronic hepatitis B virus. In PBMCs from 11 of 12 individuals with undetectable HIV-1 RNA in plasma, GS-9620 activated viral RNA expression an average of 5.8-fold. (range, 2-fold to 26.8-fold across donors). The ability to induce HIV expression was reduced in subsequent treatments with GS-9620. In an extension of these observations, Whitney and colleagues (Abstract 108) examined the impact of a TLR7 agonist

on plasma viremia in SIV_{mac251}-infected animals that were virologically suppressed on antiretroviral therapy. After 45 weeks of virologic suppression, macaques were given repeated doses of a TLR7 agonist at twice-monthly intervals while taking antiretroviral therapy. Cell-associated viral DNA was quantified in PBMCs and in colon and lymph node biopsies taken before and after completion of treatment with the TLR7 agonist. Although there was no obvious effect on plasma viremia after the first 3 doses of the TLR7 agonist, there were transient and consistent increases in plasma viremia (500 copies/mL-1000 copies/mL) between doses 4 and 7. Further, there were substantial reductions in viral DNA content in all tissue samples and a lower viral set point after cessation of antiretroviral therapy. These exciting findings pave the way for studies to assess the impact of TLR7 agonism on viral reservoirs in HIV-1-infected individuals taking effective antiretroviral therapy. 

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

All cited abstracts appear in the CROI 2015 Program and Abstracts eBook, available online at www.CROIconference.org.

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