Invited Review

CROI 2018: Advances in Basic Science Understanding of HIV

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The conference on Retroviruses and Opportunistic Infections represents the most important venue for the dissemination of research advances in HIV and AIDS. The 25th conference, held in Boston, featured presentations that provided insight into the mechanisms of HIV-1 spread in tissues as well as new information on mechanisms of HIV-1 persistence in individuals on effective antiretroviral treatment. The ability of the conference to convey research findings for a general audience is enhanced, to a large part, by preconference workshops. These workshops feature leading researchers who aim to present cutting edge research to a general audience. These sessions rank highly in terms of education and professional value.

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Virology

Bjorkman (Abstract 4) discussed studies employing electron tomography to visualize the spread of HIV-1 in tissues. Electron tomography is a variation on 2-dimensional electron microscopy in which 2-dimensional projections of objects of interest, such as viral particles, are visualized on a grid. In electron tomography, various views of objects are recorded using a tilting microscope. This allows 2-dimensional images of an object to be taken at different angles from which a 3-dimensional image of the object can be created. Bjorkman's group used the humanized mouse model to examine HIV-1 infection within bone marrow of these mice.

At least 3 mechanisms for HIV-1 dissemination were found. The first was classic HIV-1 budding from infected CD4+ T cells.

The second involved synaptic transmission by uropods. Although a number of groups have described synaptic virus transmission in which transfer of virus from an infected cell to a target cell occurs through intimate synaptic structures formed between the 2 cells, Bjorkman's study focused on transmission through uropods that are cellular protrusions that form synaptic contacts with other cells. Synaptic transmission of murine leukemia virus (MLV) was first observed by Mothes group.[1] HIV-1 transmission was observed by uropods that contain microvesicles and organelles, which distinguished them from uropods of macrophage origin that are devoid of organelles and vesicles. Uropods appeared to be facilitating transfer of virus particles from infected donor cells to the uninfected target cell. Uropods with budding HIV particles were also found to originate from infected macrophages to facilitate infection of CD4+ T cells.

The third mechanism for HIV-1 transmission that was revealed by electron tomography involved macrophages. Recent studies from the Benichou laboratory described the transfer of virus from CD4+ T cells to macrophages in culture and macrophages infected in this way were found to produce new virus within hours.[2] When macrophages were visualized by electron tomography, mature virus particles were observed budding from the cell surface. However the majority of viral particles appeared in membranes in closed compartments inside the cell. Further analysis revealed that these compartments were not contiguous with the extracellular environment. Although these compartments were enclosed, they had the capacity to fuse with surface invaginations that offered a pathway for virus release from the infected macrophage. This appears to be a novel mode of HIV-1 production that poses many questions, some of which are relevant to the issue of viral reservoirs.

Previous studies have demonstrated the presence of HIV-1 variants within intracellular vesicles of infected macrophages, that those vesicles were contiguous with the extracellular space and further, that those vesicles retained HIV-1 in an infectious form for extended intervals. Therefore, it remains to be determined whether this mode of HIV-1 manufacture in which particles are contained within an enclosed compartment, offers a novel mechanism that allows the virus to persist in an infectious form. Furthermore, this process is likely to bypass the constraints conferred by HIV-1 tropism in which macrophage infection requires a viral envelope with a high affinity for CD4. Viral variants budding into enclosed compartments that were derived from an engulfed CD4+ T cell would exhibit T cell tropism even though they are budding from macrophages.

Mechanisms of Viral Persistence

The majority of presentations in the basic science category focused on mechanisms of viral persistence in the face of effective antiretroviral therapy (ART). At least 3 mechanisms have been implicated in maintaining HIV-1 persistence in infected individuals. Most of the attention has focused on viral latency in which the infected cell harbors an integrated provirus in a transcriptionally silent state. As the provirus is silent, there is little to distinguish the latently infected cell from an

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uninfected cell. As such, this makes identification and elimination of latently infected cells one of the major challenges facing the HIV-1 cure field. Numerous laboratories have been investigating ways to reactivate the latent reservoir so as to expose the latently infected cell to host immune clearance and additionally, to promote clearance of the infected cell by viral cytopathicity.

A second mechanism proposed to maintain viral persistence involves the homeostatic maintenance of infected cells. If an infected cell undergoes mitosis, the provirus would be duplicated between the daughter cells thereby increasing the number of cells harboring a viral genome. A third and more controversial mechanism involves generation of newly infected cells by de novo viral infection or, as commonly referred to, ongoing viral replication. This terminology creates confusion and angst amongst cure researchers. From a virologic standpoint, ongoing viral replication involves infection of a cell that generates progeny virus that then go on to infect a new cell. Through successive rounds of replication, the virus spreads through the cell population. This is distinguished from single-cycle or limited rounds of infection in which an infected cell generates progeny virions that infect a new cell, but the newly infected cell does not repeat the process. Under conditions of single-cycle infection or limited rounds of infection, there would be less opportunity for viral evolution and development of antiretroviral drug resistance. The term active reservoir is more frequently being used in the HIV cure field to describe infected cells that are discernable by the presence of viral transcripts. Previous studies have indicated that the absolute level of cell-associated viral RNA in HIV-1–infected individuals on suppressive ART predicts the time to viral rebound when treatment is interrupted.[3] This suggests that at the least, cells actively transcribing HIV-1 comprise a biologically active component of the viral reservoir.

Abstract 66 attempted to define the timing of reservoir establishment in acute HIV infection using HIV RNA-positive cells as surrogates of the reservoir. The investigators obtained inguinal lymph node biopsy from acutely infected individuals who initiated ART in Fiebig stages 1 and 2 versus Fiebig stages 3 to 5. Reservoirs were characterized by in situ hybridization to identify viral RNA- and viral DNA-positive cells. Several important observations emerged from this analysis, including that viral DNA-positive cells were established as early as Fiebig stage 1, and the frequency did not change after 48 weeks of ART. Viral RNA-positive cells were also established as early as Fiebig stage 1 and the higher frequency of these cells in Fiebig 1 was associated with fewer HIV-1 specific CD8+ T cells.

The investigators also documented damage to lymph node architecture at the earliest stage of acute HIV-1 infection as indicated by deposition of collagen. Therefore, damage to lymph node architecture could be observed as early as Fiebig stage 1. However, although initiation of ART in Fiebig 1 did not alter the frequency of viral DNA-positive cells, it was able to improve lymph node architecture. These findings are in general agreement with studies from the Ananworanich laboratory, presented at CROI 2017 (Abstract 124), that very early initiation of ART does not substantially delay time to viral rebound when treatment is interrupted. Collectively, these studies suggest that uncontrolled viral replication in the absence of cell-mediated immunity leads to rapid reservoir establishment and this obfuscates attempts to reduce reservoir size and increase possibility of viral eradication through very early initiation of ART. These studies are somewhat at odds with earlier studies[4] indicating that treatment in the acute phase promoted more effective control of the number of cells harboring reactivateable HIV-1 (15809898).

Although early initiation of ART appeared to have limited impact on viral reservoir size in treated adults, the situation appears to be different in children. Abstract 135 examined 2 cohorts of Thai children, one of which was on prophylactic ART and the second cohort initiated ART within 6 months of age. Viral reservoir measurements included total levels of HIV-1 DNA, integrated HIV-1 DNA, and inducible HIV-1 (TILDA). Viral load strongly correlated with levels of total integrated HIV-1 DNA as well as the frequency of cells harboring inducible virus. Importantly, levels of viral DNA and in particular, levels of inducible HIV-1, were markedly reduced in children who started ART before 6 weeks of age versus in children initiating ART after 6 weeks. Therefore, very early ART in the pediatric population dramatically reduces viral reservoir size. Similarly, in Abstract 136, children treated in the first week of life had very low viral reservoir size after 84 weeks of ART and interestingly, the majority of proviruses present in the reservoir by the second year of life were defective, suggesting that early treatment limited the maintenance of the biologic active reservoir.

This theme was continued in Abstract 67 where the investigators characterized early immune responses and reservoir activity in female participants with acute HIV-1 infection. There was a strong correlation between viral burden and the breadth of CD8+ T cell responses in contrast to what was presented in Abstract 66. However, early ART initiation led to a decrease in total HIV-1 DNA levels at 1 month and 12 months. Despite this, in some individuals there was a considerable amount of HIV-1 Gag p24 in lymph germinal centers despite complete suppression of plasma viremia. Although it is unclear whether the viral antigen was being produced in infected cells or whether it simply represented virions trapped on the cell surface, this indicates that analysis of peripheral blood does not provide accurate insight into ARV response in lymphoid tissue.

There has been a lot of attention regarding the role played by homeostatic duplication of integrated proviruses in the maintenance of HIV-1 persistence under ART suppression. Proviruses can be duplicated through the normal process of cell proliferation. However, some clones are overrepresented in the population because they are duplicated more frequently. This occurs if the provirus integrates close to a gene involved in cell cycle control. In this situation, normal transcriptional regulation of the cell cycle control gene is interrupted by the juxtaposed HIV-1 transcriptional unit. This leads to uncontrolled cell proliferation and continuous rounds of proviral duplication. Although provirus insertions close

to genes involved in cell cycle regulation are frequently observed, there is, surprisingly, little evidence that this process can drive T-cell up abnormalities such as leukemia.

Since the demonstration of clonal expansion in HIV-1 infected individuals, it was initially suspected that proviruses expanded through clonal proliferation would predominantly be defective since the process of duplication would be expected to lead to proviral activation and production of viral proteins that could target the cell for immune clearance or cytopathicity. However, work in the last 2 years has demonstrated that functional proviruses can be expanded in this way and serve as a template for infectious virus production that can be detected in the plasma of HIV-1 infected individuals. Studies presented in Abstract 68 provided some insight into the mechanism, through which clonal expansion can occur without activation of the host cell, and further expanded on the theme that the reservoir of expanded clones is dynamic. The investigators followed up on recently published studies demonstrating that a substantial proportion of latent proviruses are generated by clonal expansion. This, by definition, argues that the process of clonal expansion does not lead to viral reactivation to an extent that would result in host immune clearance or viral cytopathicity. Therefore, the investigators set out to determine the process that can drive clonal expansion of latent viruses without triggering activation of the infected cell. The investigators demonstrated that CD4+ T cells containing replication-competent virus can proliferate in response to T-cell receptor activation and cytokine treatment (eg, with interleukin 7 treatment). Although this has been observed previously in the presence of the chemokine CCL19,[5] cytokine-driven proliferation of cells carrying replication-competent virus did not result in virus production. The investigators then explored the dynamics of the clonal population of latent viruses that are maintained in HIV-1 infected individuals. Virus was recovered from 8 individuals on suppressive ART at various intervals over a 2-year period. Proviruses in those cells were then characterized by single cell sequencing. Some clones comprised a large percentage of the viral population at various time points and others only prevailed at individual time points, suggesting that some clones fluctuate in their abundance with time and that this waxing and waning can occur over a period of years. Analysis of the residual viremia in these individuals was also reflected in the residual virus population where viruses matching expanded clones appeared and disappeared with similar dynamics. This indicates that clonal expansion of latent virus can be accompanied by release of virus particles but that duplication of proviruses during cell proliferation can occur in response to cytokines and occur without infectious virus production. As such, this process represents an important mechanism for maintenance of the viral reservoir and poses a formidable challenge to efforts to eradicate HIV-1.

Given the ability of proviruses to be maintained by homeostatic proliferation, there remains a question on the value of proviral DNA as a surrogate with which to gauge reservoir activity and more importantly, to monitor the effect of therapeutic strategies aimed at reducing viral reservoir size. Abstract 69LB presented results on cell-associated HIV-1 DNA measurements at 3 time points over a 3- to 10-year period in more than 1000 infected individuals. Levels of viral DNA decreased steadily over the 10-year period and differences in the levels of cell associated viral DNA between individuals diminished over this time. The slope of viral decay indicated a half-life of between 5 and 11 years for approximately 75% of the individuals. However, despite being on effective therapy, there was no depreciable DNA decay in 25% of the participants. Viral reservoir size, as measured by cell-associated DNA levels, was smaller in individuals who initiated treatment earlier. The investigators also examined the relationship between viral blips and reservoir size. Blips were associated with higher level of cell-associated viral DNA and predicted the decay of the viral reservoirs indicating that blips are a biologically relevant surrogate of the viral reservoir.

The issue of viral reservoir replenishment through ongoing or de novo infection continues to be a hotly debated topic at CROI. Abstract 70 examined proviral genetics in the infected individual prior to and after 2 to 13 years of suppressive ART in paired lymph node and peripheral blood samples. Analysis of integration sites in cells from lymph nodes and peripheral blood suggested that HIV-1 persistence was maintained by the homeostatic proliferation of cells infected prior to ART initiation and not by viral replication in peripheral blood or in lymph nodes. It will be important to determine whether the viral population that rebounds when treatment is interrupted has a genetic composition similar to the population of expanded proviruses. As alluded to earlier, this analysis does not exclude the generation of newly infected cells through single or low cycles of de novo infection. Abstract 71 examined the impact of treatment intensification with the integrase strand transfer inhibitor (InSTI) dolutegravir on episomal viral DNA levels. Previous studies demonstrated that intensification of ART with InSTIs led to a rapid and transient increase in levels of episomal viral DNA in approximately 30% of individuals, and especially for individuals on protease inhibitor (PI)-based regimens.[6,7] Such an outcome can only occur if there were cells in those participants in the process of being infected with the HIV-1. Those earlier studies suggested that a sizeable percentage of infected individuals have some degree of de novo infection even under suppressive ART. Those observations were further underscored by changes in immune inflammation markers such as D-dimer or frequencies of activated CD8+ T cells. Dolutegravir has profound antiviral efficacy to the extent that resistance to dolutegravir is far less common than that observed with individuals on other InSTIs. In contrast to the earlier reports, the investigators saw no significant changes in the frequencies of episomal viral DNA, even though the study was powered to observe as little as 3-fold differences in episomal DNA frequency. Furthermore, there were no changes in T-cell activation status or in plasma markers of inflammation. The reason for the differences between this and earlier studies is unclear. Acute changes in episomal DNA numbers were observed selectively in individuals on PI-based regimens and those individuals were underrepresented in the current study. In addition, earlier studies were

conducted with the InSTI raltegravir, which shows considerable penetration to gut-associated lymphoid tissue (GALT) where most infection events are likely to occur. Therefore it is unclear whether the differences in the studies represent differences in the population or differences in the pharmacokinetic characteristics of raltegravir versus dolutegravir.

Viral Reservoir Elimination Studies

One of the major challenges facing HIV-1 cure researchers is a lack of host cell markers that can be used to identify infected cells in individuals on effective ART. Studies published in the past 2 years[8] identified CD32a as being selectively expressed on CD4+ T cells harboring latent, replication-competent proviruses. This was further expanded in Abstract 155 where CD32+/PD1+ T follicular helper T cells were found to be the major HIV reservoir in individuals on suppressive ART. Follicular helper T cells have previously been shown to have the highest levels of HIV-1 DNA among the CD4 T cell subsets, and additionally, were found to have higher levels of cell-associated viral transcripts in aviremic individuals.[9] However, although high levels of viral DNA and viral transcripts were found in lymph node CD32a+ and in PD1+ CD4+ T cells, cells expressing both markers were found to harbor the highest levels of viral transcription. The authors concluded that CD32 was neither a specific marker for the latent HIV-1 reservoir nor expressed exclusively in HIV-1–infected cells. Three additional abstracts (Abstracts 156, 157, and 158) presented data that further called into question CD32 as a reservoir marker. For example, studies presented in abstract 156 used viral outgrowth assays to determine the size of the latent reservoir in CD32-positive and CD32-negative cells and concluded that the majority of latently infected cells were CD32a negative. Similarly, in abstract 157, viral outgrowth assays showed a lack of association between the amount of replication-competent HIV and the frequency of CD32a+ cells. Furthermore, the point was made that CD32 is expressed on naive T cells and numerous lines of investigation have shown that the latent reservoir resides predominantly in memory CD4 T cells. CD32+ cells were found to be highly activated, which would be biologically inconsistent with maintenance of viral latency. Finally, abstract 158 represented evidence that CD32+ cells were transcriptionally active for HIV-1 rather than latently infected. However, although CD32 expression did not selectively correlate with HIV-1 or simian immunodeficiency virus (SIV) infection in CD4+ T cells in blood or tissues, there was a positive correlation between cell-associated viral DNA and RNA and the frequency of CD32+ cells. Collectively, these studies indicate that CD32a is not a marker of latently infected CD4+ T cells. However, it does appear to be co-expressed with viral RNA in lymph nodes of HIV-1–infected individuals on effective ART. Further studies will be required to determine whether cell-associated RNA reflects a latent reservoir as it exits and whether CD32a can be used to target those cells as they exit. As discussed above, it is possible that the latent reservoir is not static but goes through intermittent intervals of reactivation.

One strategy being explored for elimination of the latent reservoir centers on reactivation of the reservoir, thereby rendering the infected cell to viral cytopathic effect or to cell mediated immune clearance. Several agents have been shown to effectively reactivate HIV-1 latency in cells from infected individuals ex vivo without overtly triggering cellular activation. Many latency reactivating agents under investigation are histone deacetylase inhibitors that promote relaxation of chromatin, which facilitates the interaction of cellular transcription factors with the HIV-1 LTR to facilitate proviral transcription. Romidepsin is one of the most potent histone deacetylase inhibitors for the reactivation of HIV-1 ex vivo. Because it is already approved by the US Food and Drug Administration (FDA) for the treatment of T-cell lymphoma, it has been rapidly explored as a latency reactivating agent in infected individuals. However, studies conducted to date with romidepsin and other histone deacetylase inhibitors have been underwhelming; the extent to which they reactivate HIV-1 appeared modest. Abstract 72 examined whether a single romidepsin infusion activated HIV-1 transcription in infected individuals on effective ART. Induction of HIV-1 expression was assessed from changes in plasma viremia using a single copy HIV-1 RNA assay as well as from changes in cell-associated HIV-1 RNA. Following romidepsin administration, the in vivo impact of romidepsin on histone acetylation was assessed from the frequency of CD4 T cells with activated NF-κB as well as histone acetylation levels. Single romidepsin infusions, at concentrations shown to reactivate HIV latency ex vivo, had no impact on plasma viral RNA levels nor cell-associated viral DNA or RNA. Nevertheless, there was a significant increase in T cell activation at the highest dose of romidepsin. These studies contradict earlier published work that numerous romidepsin infusions increased plasma viral RNA levels in HIV-1 infected individuals on effective ART. Study design differences may explain the opposing results, such as single dose versus numerous doses of romidepsin.

On a more positive note, Abstract 73LB presented results that combination of a broadly neutralizing antibody with a TLR7 agonist delayed viral rebound in acutely ART-treated, simian-human immunodeficiency virus (SHIV)-infected macaques. GS-9620 is a TLR7 agonist with the ability to stimulate innate immunity. Studies presented at CROI 2017 (eg, Abstract 338LB) demonstrated that the TLR7 agonist GS-9620 promoted prolonged viral control in infected macaques and furthermore, prevented viral rebound in approximately 50% of infected animals. Abstract 73LB examined the combined effects of the broadly neutralizing antibody PGT121 together with TLR7 administration on viral rebound kinetics and on virologic control. All control animals rebounded within 1.5 months of ART discontinuation and no significant difference was observed with animals receiving the TLR7 agonist alone. There was a 5-fold delay in time to rebound for half the animals receiving antibody and agonist and 50% of the animals showed no viral rebound by day 168. Furthermore, adoptive transfer of lymphocytes from non-rebounders to naive animals failed to transfer infectious virus. The synergistic impact

of combined PGT121 and GS9620 is possibly due to activation of the CD4 T cell reservoir by GS-9620 followed by enhanced clearance of reactivated virus by PGT121. Although these findings are important, one has to keep in mind that these effects were observed in monkeys in which ART was initiated at week 1 and that used an SHIV variant that is highly neutralization sensitive. It remains to be determined whether similar levels of efficacy would occur in chronically infected animals who initiate ART later in their infection course.

All cited abstracts appear in the CROI 2018 Abstracts eBook, available online at www.CROIconference.org

Financial affiliations in the past 12 months: Dr Stevenson has no financial conflicts to report for the past 12 months

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