Invited Review

CROI 2016: Basic Science Review

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The 2016 Conference on Retroviruses and Opportunistic Infections continued to maintain balance in the representation of different areas of research related to HIV/AIDS. The basic science category encompasses research on viral reservoirs and HIV cure, on cellular factors regulating the interplay between virus and host, and on factors that influence viral pathogenicity. Basic research on factors that influence the interaction between the virus and the host cell continues to unearth surprises with the identification of a new host antiviral factor. Further, research into the mechanisms of viral persistence reveals that there is much to learn about how HIV-1 is able to persist in the face of antiviral suppression.

Keywords: CROI, 2016, cure, viral reservoir, persistence, posttreatment control, cofactors, reservoir elimination, virology, pathogenesis

Viral Reservoirs and Persistence

As the research field embarks on finding a cure for HIV-1 infection, there has been substantial effort made in understanding how the virus is able to persist in the face of suppressive antiretroviral therapy, as was discussed at the 2016. Conference on Retroviruses and Opportunistic Infections (CROI). Although the level of viral suppression is profound and can be sustained for years, there is rapid recrudescence of plasma viremia shortly after treatment is interrupted. A stark illustration of this was provided in a study by Henrich and colleagues.[1] In this study, 2 HIV-infected individuals underwent reduced-intensity conditioning allogeneic stem cell transplantation and were transfused with donor cells that had normal CC chemokine receptor 5 (CCR5) alleles. Following transplant, HIV-1 DNA and RNA were undetectable in circulating CD4+ cells and plasma during suppressive antiretroviral treatment. Further, viral DNA was undetectable in gut-associated lymphoid tissue in 1 individual. Both individuals underwent an analytic treatment interruption and became viremic after 84 days and 225 days, respectively. In both cases, rebound in viremia was substantially delayed when compared with typical rebound of 1 week to 8 weeks after the interruption of antiretroviral therapy. The source of rebounding virus in these individuals is a topic of great speculation. There appeared to be a complete replacement of host CD4+ T cells (microchimerism testing indicated less than 0.001% of peripheral blood cells were of host origin). This suggests that there may be nonlymphoid reservoirs that maintain viral persistence in the face of suppressive antiretroviral therapy. Further studies of this kind are needed to reveal the long-lived cellular reservoirs that sustain HIV-1 in the face of viral suppression. In contrast to the outcome of this study, sustained HIV-1 remission was achieved by mild myeloablative allogeneic hematopoietic stem cell transplantation using donor cells that were homozygous for a 32–base pair deletion in CCR5 (the Berlin patient, Timothy Brown).[2] The difference here is that viruses emerging from any long-lived host reservoirs would be unable to encounter permissive host cells. This has emboldened the field of HIV research to devise strategies with which to achieve sustained remission without resorting to allogeneic stem cell transplantation.

The debate continues as to whether viral reservoirs that persist in the face of antiretroviral therapy are quiescent or whether a component of a viral reservoir can harbor the virus in an active state. The general consensus is that a reservoir that persists in the face of therapy predominantly comprises latently infected memory CD4+ T cells. Because the virus is in a latent state, it is believed that there are no viral proteins expressed within the latently infected cell that can render the cell vulnerable to killing by cytotoxic T cells. This provides the rationale for cure strategies that promote reactivation from latency, so as to render infected cells susceptible to attack by cytotoxic T cells or viral cytopathic effects.

Are viral reservoirs that persist under antiretroviral therapy quiescent or part of a reservoir harboring virus in an active state?

Data from Cartwright and colleagues (Abstract 22) challenged the notion that the viral reservoir is quiescent during suppressive antiretroviral therapy. The investigators examined whether depletion of CD8+ T lymphocytes would impact persistent viral reservoirs in simian immunodeficiency virus (SIV)-infected rhesus macaques receiving suppressive antiretroviral therapy. Thirteen rhesus macaques were infected with SIV_{mac239} and were placed on a 4-drug antiretroviral regimen 8 weeks postinfection. On entering an aviremic state, CD8+ T cells were depleted following intravenous administration of anti-CD8 antibody. On depletion of CD8+ T cells, there was a rapid recrudescence of plasma viremia in all treated animals. Viremia resolved on repopulation of CD8+ T cells, and this was found to be independent of repopulation

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by CD8+ natural killer cells. The investigators also demonstrated that the level of viremia following depletion of CD8+ T cells correlated with the number of SIV-specific CD8+ T cells prior to depletion.

This study provides intriguing evidence that, in the setting of suppressive antiretroviral therapy, a portion of the viral reservoir maintains the virus in an active state and that antiretroviral therapy and suppression of CD8+ T-lymphocytes cooperate to effect suppression of virus. This study underscores the rationale for the use of therapeutic vaccines in reservoir elimination, as well as checkpoint inhibitors to boost CD8+ T-cell activity in HIV-1–infected individuals taking suppressive antiretroviral therapy.

CD8+ T cells suppress viral reservoir activity during suppressive antiretroviral therapy.

A study from Lifson, Picker, and colleagues suggested the existence of sanctuaries of persistent HIV-1 replication based on the anatomic distribution of CD8+ T cells.[3] Data from this study indicated that B-cell follicles support persistent SIV replication, because CD8+ T cells are excluded from B-cell follicles. Folkvord and colleagues (Abstract 23) examined whether the relative compartmentalization of SIV in B-cell follicles (where CD8+ T cells are excluded) versus extrafollicular regions (where CD8+ T cells are abundant) would diminish on depletion of CD8+ T cells. Three chronically SIV-infected rhesus macaques underwent lymph node biopsy prior to and following antibody-mediated depletion of CD8+ T cells. Frequencies of SIV RNA–positive cells were determined by in situ hybridization. SIV RNA–positive cells were more abundant in follicular versus extrafollicular cells prior to depletion of CD8+ T cells (11-fold and 44-fold higher in follicular and extrafollicular cells, respectively). Further, there was an increase in SIV RNA–positive cells in extrafollicular tissue to the extent that the difference in frequencies in follicular versus extrafollicular regions normalized. This underscores previous findings that virus-specific CD8+ T cells dictate virus compartmentalization and that extrafollicular tissues, which are accessible to CD8+ T cells, are most susceptible to CD8+ T-cell suppression.

B-cell follicles, from which CD8+ T cells are largely excluded, present sanctuaries for viral replication free of the suppressive effect of virus-specific cytotoxic T lymphocytes. Given the possibility that CD8+ T cells play a key role in viral suppression, there is considerable interest in augmenting CD8+ T-cell responses in order to better suppress viral reservoir activity. One approach being explored utilizes antibodies that target the programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) axis to reverse CD8+ T-cell exhaus-tion. Abstract 25 examined the impact of anti–PD-L1 antibody (BS936559) on immunologic and virologic profiles of 6 HIV-1–infected individuals taking suppressive antiretroviral therapy. Participants received a single infusion of an anti–PD-L1 antibody. Gag-specific CD8+ T-cell responses and plasma HIV-1 RNA levels were assessed over 28 days postinfusion. Owing to strong responses in 2 of 6 individuals, there was an overall trend of increases in the average percentage of HIV-1–specific CD8+ T cells, and there were no changes in plasma viral RNA or cell-associated viral RNA over 28 days postinfusion. This represents the first study to employ a PD-1/PD-L1 axis inhibitor in HIV-infected individuals taking suppressive antiretroviral therapy. Additional studies are needed to determine whether reversal of CD8+ T-cell exhaustion can impact viral reservoirs that persist in lymphoid tissue during suppressive antiretroviral therapy.

B-cell follicles act as potential sanctuary sites for viral persistence.

Previous studies have indicated that T follicular helper (Tfh) cells are the major compartment of CD4+ T cells for HIV-1 infection and replication in viremic individuals. Abstract 82 examined whether this observation also held true for aviremic individuals. The investigators compared the amount of viral RNA produced by CXC chemokine receptor 5–positive/PD-1–negative and PD-1–positive memory CD4+ T cells isolated from blood and lymph nodes of individuals taking long-term surpressive antiretroviral therapy. Levels of viral RNA were 800- to 1200-fold higher in PD-1–positive CD4+ T cells relative to the PD-1–negative cell population in lymph nodes and 5702- to 73,000-fold higher relative to CD4+ T-cell populations in blood. It is difficult to interpret from the level of viral RNA and viral outgrowth measurements the frequency of infection with this approach. Nevertheless, these data underscore the central role played by PD-1–positive CD4+ T cells in the lymph node as a major infected cell compartment for HIV-1–infected individuals taking suppressive antiretroviral therapy.

Leth and colleagues (Abstract 26LB) examined the impact of a therapeutic HIV immunization and a latency-reversal agent on viral reservoirs in HIV-1–infected individuals taking suppressive antiretroviral therapy. In a single-arm trial, 20 HIV-1–infected adults received 6 immunizations with the Vacc-4x vaccine, a peptide-based HIV-1 therapeutic vaccine that targets conserved domains on Gag p24, followed by 3 weekly injections of romidepsin. These researchers previously demonstrated that administration of romidepsin increased both plasma and cell-associated HIV-1 RNA.[4] Following therapeutic HIV immunization and romidepsin infusion, viral reservoirs were gauged by changes in total cell-associated HIV DNA and viral outgrowth assay in 17 individuals who completed all Vacc-4X immunizations and romidepsin infusions. Cell-associated proviral HIV DNA and viral outgrowth (evaluated in 6 of 17 participants) were each reduced by 40%. As this was a single-arm study, the relative contribution of Vacc-4x vaccines versus romidepsin injections to changes in reservoir activity cannot be determined. Nevertheless, this study underscores the need for additional trials that combine therapeutic immunization with latency-reversing agents to reduce the size of the viral reservoir.

Whitney and colleagues (Abstract 95LB) presented an update on ongoing studies aimed at evaluating the impact of an investigational Toll-like receptor 7 (TLR7) agonist (GS-986) in SIV-infected monkeys receiving an antiretroviral regimen. In 2015, these researchers presented intriguing evidence that administration of an oral TLR7 agonist induced transient plasma viremia. There was also a lower viral set point after interruption of antiretroviral therapy in animals receiving repeated doses of the TLR7 agonist. At CROI 2016, the investigators examined whether GS-986 and an investigational clinical compund (GS-9620) could affect viral reservoirs in SIV-infected macaques taking surpressive antiretroviral therapy. GS-986 induces interferon-α at doses that also induce transient plasma virema. However, low or undetectable interferon-α was induced by GS-9620. The investigators determined whether the effects of TLR7 agonists on plasma viremia could be uncoupled from induction of interferon-α. Eleven SIV_{mac251}-infected macaques underwent 65 days of treatment with surpressive antiretroviral therapy and maintained virologic surpession below 50 copies/mL of SIV RNA. Animals received either GS-986 or GS-9620 once every 2 weeks while maintained on supressive antiretroviral therapy. Peripheral blood mononuclear cell (PBMC)-associated SIV DNA, DNA in lymph nodes and colon, and SIV outgrowth were assesed following stimulation with concanavalin A ex vivo. Transient but inconsistent SIV RNA blips were observed in all animals treated with the TLR7 agonist from doses 3 to 10 but not after doses 11 to 19. On completion of treatment with the TLR7 agonist and prior to termination of antiretroviral therapy, SIV DNA levels were reduced in all tissues, as was the level of SIV induction relative to the control group. The kinetics of rebound in plasma viremia in 7 of 9 treated monkeys were similar to those of the control group. Intriguingly, in 2 animals, plasma viremia was undetectable for 60 days after discontinuation of antiretroviral therapy, and they were also negative for virus reactivation ex vivo. These important results support the rationale for the use of TLR7 agonists in elimination of viral reservoirs in HIV-1–infected individuals taking supressive antiretroviral therapy.

Siliciano and colleagues previously demonstrated that the vast majority of proviruses in HIV-1–infected individuals are defective and, further, that many cells harboring intact proviruses do not readily generate infectious virus, as measured by viral outgrowth assays.[5] In Abstract 83, Bruner and colleagues examined whether the fraction of defective proviruses increased over the course of infection. The fraction of defective proviruses was determined in individuals treated during acute or chronic HIV-1 infection. Provirus sequencing was conducted using full-genome polymerase chain reaction (PCR) assay following limiting dilution, and the number of intact proviruses was compared to levels of cell-associated viral DNA and viral outgrowth measurements. Less than 5% of proviruses were found to be intact in both acute and chronic infections. This indicates that defective proviruses accumulate rapidly following acute infection and is consistent with the rapid cytopathic effect of HIV-1 that would manifest in cells harboring biologically competent proviruses. The investigators also demonstrated that the majority of proviruses are predicted to be unable to make viral protein and, as such, are unlikely to be eliminated by purging protocols. However, it remains to be determined whether strategies that eliminate functional proviruses but not proviruses that are unable to make protein would produce a functional cure (ie, one that does not eliminate all virus from the infected individual but that leaves a viral reservoir that does not rebound if the infected individual discontinues therapy).

The issue of whether the viral reservoirs that persist in the face of antiretroviral therapy are nondynamic and quiescent or whether a proportion of the viral reservoirs retains some viral activity is of great debate. For example, cell-associated viral RNA persists in many individuals taking suppressive antiretroviral therapy, and the level of cell-associated RNA has recently been shown to correlate with time to viral recrudescence following an analytic treatment interruption.[6]

Does cell-associated viral RNA act as a surrogate for viral reservoir activity during antiretroviral therapy?

Hong and colleagues (Abstract 85) investigated the fraction of cells that harbor cell-associated viral RNA and the level of cell-associated RNA in individual cells from untreated individuals, viremic individuals, and aviremic individuals taking suppressive antiretroviral therapy. Unspliced viral RNA was examined by extracting RNA from multiple PBMC aliquots followed by dilution to end point or by cDNA synthesis and sequencing of cDNA molecules via single-genome sequencing and, additionally, serial dilution of PBMCs followed by measurement of HIV DNA and unspliced cell-associated viral RNA.

The frequency of proviruses expressing unspliced, cell-associated RNA was the same in viremic and aviremic individuals. In contrast, cells from viremic individuals had higher levels of cell-associated RNA. Because identical cell-associated RNA sequences were identified in multiple PBMC aliquots from aviremic individuals, this suggests that unspliced viral RNA is commonly expressed by expanded CD4+ T-cell clones. The investigators hypothesized that the higher level of cell-associated RNA in viremic individuals reflects a state of active viral replication, compared with cells obtained from aviremic individuals. An alternative hypothesis is that the high level of immune inflammation in viremic individuals creates conditions for more active transcription of proviruses. The authors also hypothesized that CD4+ T cells expressing unspliced viral RNA may contribute to viral recrudescence on interruption of antiretroviral therapy. It is unclear whether the cells that actively express unspliced HIV RNA in aviremic individuals are similar to those in latent viral reservoirs that persist in the face of suppressive antiretroviral therapy. It will also be interesting to determine to what extent cells that express unspliced HIV RNA contribute to the level of inducible virus that is measured using viral outgrowth assays.

As clinical studies aimed at eliminating long-lived viral reservoirs are developed, there is considerable interest in targeting individuals who initiated antiretroviral therapy early after infection. Such individuals may have less extensive viral reservoirs. Consistent with this, there is evidence that some individuals who initiate therapy during primary HIV infection exhibit virologic remission when treatment is halted. Gossez and colleagues (Abstract 87) examined the extent of post-treatment control in a cohort of individuals from South Africa and Uganda who initiated antiretroviral therapy during primary HIV infection and then subsequently underwent a treatment interruption. One hundred thirty-seven individuals within the first 6 months of seroconversion were recruited and randomly assigned to no treatment or 4 weeks to 8 weeks of antiretroviral therapy. Time to viral rebound following treatment interruption was compared with data from a previous UK cohort recruited under a similar protocol. Approximately 23% of those in the African study maintained an HIV RNA level below 400 copies/mL for 188 weeks following treatment interruption. This was a statistically significantly greater interval of viral remission than was observed among participants in the UK trial. Levels of viral DNA at treatment interruption were a predictor of time to rebound especially among UK participants. Given the substantial percentage of individuals who underwent remission following treatment interruption, this important study underscores the rationale for functional virologic cures that focus on individuals treated during primary HIV-1 infection.

"Shock-and-kill" protocols employ small molecules that are designed to reactivate HIV-1 production so that the host cell can be eliminated via viral cytopathic effects or CD8+ T-cell–mediated clearance. A number of small molecules including transcriptional activators and chromatin remodelers have been explored for the ability to reactivate latent HIV-1 in vitro and in vivo. Abdel-Mohsen and colleagues (Abstract 81) presented evidence that galectin-9 promotes reactivation of latent HIV-1. Galectin-9 is a β-galactosidase–binding lectin that exhibits a variety of biologic effects including tissue inflammation, T-cell immune exhaustion, and induction of apoptosis. Recently, galectin-9 was shown to be rapidly produced during acute HIV-1 infection and to remain at high levels during suppressive antiretroviral therapy.[7] Galectin-9 was also shown to augment expression of the cyclin-dependent kinase inhibitor p21, and it was shown that p21 regulates HIV-1 transcription.[8] Given this background, the investigators examined whether the impact of galectin-9 on p21 might lead to a reactivation of viral latency. The level of cell-associated HIV-1 RNA correlated with endogenous levels of plasma galectin-9. When galectin-9 was added to CD4+ T cells from HIV-1–infected individuals, there was an average 7-fold increase in level of cell-associated HIV-1 RNA, which was statistically significantly higher than for vorinostat-treated cells. Galectin-9 also induced the expression of apolipoprotein B mRNA editing enzyme catalytic subunit 3G (APOBEC3G), which reduced infectivity of nascent virus. Galectin-9 represents an attractive agent for reservoir elimination in that it can reactivate latent HIV and induce the expression of the antiviral restriction APOBEC3G. With this dual activity, any viruses produced from the reactivated cell would be rendered noninfectious after packaging of APOBEC3G in virions.

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Virions trapped on follicular dendritic cells may represent another obstacle to HIV eradication.

A reservoir of latently infected memory CD4+ T cells is considered the single biggest obstacle to the elimination of HIV-1. However, it is unlikely to be the only obstacle, and it is possible that other reservoirs maintain viral persistence in the face of suppressive antiretroviral therapy. Carroll (Abstract 128) proposed that virions associated with follicular dendritic cells (FDCs) maintain infectivity for extended intervals and that these extracellular virions may constitute a viral reservoir for many months. Smith and colleagues demonstrated that when regular nonhumanized mice were passively immunized with an HIV-1 envelope antibody and subsequently injected with HIV-1, infectious virus could be recovered from lymph nodes 9 months later.[9] This remarkable study has gone relatively unnoticed, perhaps because of the difficulty in creating this scenario in a more physiologic setting. In support of this early study, Carroll presented evidence that human FDCs isolated from the lymph nodes of virologically suppressed individuals taking antiretroviral therapy harbored HIV-1 in a nondegradative compartment from which it could be transmitted to uninfected CD4+ T cells in vitro. Further, HIV-1 virions could be purged from FDCs with a soluble complement receptor. These studies present an extremely disconcerting scenario for the enterprise of an HIV cure. Complement-opsonized viral particles are taken up by FDCs and retained in an infectious form for extended intervals. Current strategies such as shock and kill would not impact the FDC reservoir. It is also to be expected that during uncontrolled infection, the extent of virion production could lead to massive deposition of infectious particles in FDCs, making a tough obstacle to eliminate.

HIV Virology and Pathogenesis

The replication of HIV/SIV is opposed by several cellular proteins, known as antiviral restriction factors. The most studied of these antiviral restriction factors include the cytidine deaminase APOBEC3G, tetherin/BST-2, and SAM domain and HD domain 1 (SAMHD1). APOBEC3 proteins are packaged within virions, and on infection, APOBEC3 promotes catastrophic G-to-A hypermutations in nascent viral complementary DNA. As a result, hypermutated genomes are degraded or incapable of producing functional viral components. Tetherin/BST-2

inhibits the detachment of virions from the surface of the virus-producing cell, thereby preventing it from establishing infection in a new target cell. SAMHD1 is a deoxyribonucleotide triphosphate (dNTP) hydrolase that reduces cellular dNTP to levels that are suboptimal for reverse transcription. These antiviral restriction factors have a profound impact on viral replication, and as a consequence, HIV and SIV have evolved counter defenses to obviate these obstacles to replication. The so-called viral accessory proteins all, in some way, counteract these antiviral restriction factors: APOBEC3 proteins are neutralized by the accessory protein Vif, which promotes proteasomal degradation of APOBEC3 proteins; tetherin is directed away from sites of virus assembly by Vpu; and SAMHD1 is neutralized by Vpx, also through facilitated proteasomal degradation.

Wu and colleagues (Abstract 136) presented evidence for a new antiviral restriction factor that is antagonized by the Nef protein. Nef has been shown to augment viral infectivity in a variety of cell models, but the mechanism for the enhancement of infectivity has not been defined. Two recent studies demonstrated that the host cell proteins serine incorporator 3 and serine incorporator 5 (SERINC3 and SERINC5) are inhibitors of HIV-1 infectivity and that Nef counteracts these antiviral effects.[10,11] Nef has been shown to be crucial for viral replication and pathogenicity in the SIV/macaque model, and individuals infected with Nef-deleted viruses exhibit a nonprogressive phenotype. How Nef mediates these effects has, despite 2 decades of research, not been defined. A number of studies noted an effect of Nef on viral infectivity that was relatively mild and depended on the nature of the cell from which virions were being produced. However, a clone of the CD4+ T-cell line Jurkat (E6.1) produced virus particles that were up to 100-fold less infectious in the absence of Nef.[12] Analysis of some of the cellular factors required for the infectivity in Jurkat (E6.1) cells indicated that infectivity enhancement may occur through host cell endocytic trafficking.

A possible mechanism for the impact of Nef on virion infectivity has been identified.

Further, the Moloney murine leukemia virus glycoGag protein, although unrelated to Nef, can also enhance HIV-1 infectivity. Proteomic analysis of HIV-1 virions produced in the presence or absence of Nef indicated that SERINC3 and SERINC5 were more abundant in virions produced in the absence of Nef. SERINC3 and SERINC5 are highly conserved from yeast to mammals and are predicted to contain between 10 and 12 transmembrane domains. Data presented at CROI 2016 underscored the finding that SERINC3 AND SERINC5 proteins are necessary and sufficient for the effect of Nef and glycoGag on HIV-1 infectivity. For example, inhibition of SERINC3 and SERINC5 expression eliminated the infectivity enhancement by both Nef and glycoGag, and the infectivity of Nef-deficient virions increased more than 100 fold when produced from cells lacking SERINC3 and SERINC5 expression. The precise mechanism through which Nef interacts with SERINC3 and SERINC5 is being investigated, but one possibility is that Nef promotes a redistribution of SERINC3 and SERINC5 from the plasma membrane to an intracellular compartment. Similarly, it is still unclear how SERINC3 and SERINC5 proteins inhibit particle infectivity, but it is possible that these proteins may impair the fusogenicity of virions. A central question is whether the impact of Nef on SERINC3 and SERINC5 accounts, in totality, for the requirement of Nef in viral replication in vivo. This will require identification of mutations in Nef that abrogate interaction with SERINC3 and SERINC5 proteins and of whether the mutants recreate the phenotype of viral variants deleted in Nef.

Abstract 139 provided new insight into the process through which HIV-1 gains access to the nucleus. Primate lentiviruses, including HIV/SIV, differ fundamentally from animal oncoretroviruses in that they are able to infect nonmitotic cells. For this reason, lentivirus vectors are used for transduction of nondividing target cells. During infection of the cell, viral nucleic acids are deposited in the cytoplasm of the newly infected cell in the form of a high-molecular-weight subviral complex, commonly referred to as the preintegration complex. This complex contains viral nucleic acids in association with viral enzymes, including reverse transcriptase and integrase, conferring on this nucleoprotein complex a molecular size approximating that of a ribosome. Viral reverse transcription proceeds within this complex, which must translocate to the nucleus to permit integration of virus with host cell DNA.

One of the mysteries in lentivirus biology is how this nucleoprotein complex is able to circumvent the nuclear envelope that is present in a nondividing cell. Frauke Christ and colleagues (Abstract 139) used HIV-1 virions, in which a fluorescently labeled integrase had been incorporated *in trans,* to follow conformational changes in integrase that occurred during nuclear transport of viral preintegration complexes. Fluorescence resonance energy transfer microscopy was then used to gauge the number of integrase molecules within a single preintegration complex, as well as conformational changes in the complex that accompanied nuclear entry. The investigators presented evidence that the number of integrase molecules in each preintegration complex was substantially reduced during nuclear entry. These investigators previously demonstrated that the host factor lens epithelium-derived growth factor p75 (LEDGF/p75) interacts with integrase to serve as a cofactor for integration.[13] On gaining access to the nucleus, integrase interacted with LEDGF/p75, which produced a rearrangement in the preintegration complex to allow integration. Collectively, these data indicate that the preintegration complexes undergo size reduction that enables them to pass through the nuclear membrane and further suggest that the nuclear pore may act as a molecular filter that exerts a sieving effect such that complexes that have undergone a specific adjustment in size are competent for nuclear entry. These studies are important for the design of novel antiviral agents that block the translocation of the virus to the nucleus.

The macrophage-microglial cell is the predominant if not exclusive virus-infected cell in the central nervous system

(CNS). How HIV-1 accesses the CNS is a topic of debate. It has been suggested that perivascular cells are infected in the periphery and subsequently migrate to the CNS, and it has also been suggested that trafficking of immune cells to the CNS may facilitate HIV entry. Alvarez (Abstract 141) presented evidence that SIV-infected brain macrophages can leave the CNS. The investigators introduced fluorescent superparamagnetic iron oxide nanoparticles (SPIONs) into the cisterna magna where they were taken up by phagocytic cells. Seven days after introduction of SPIONs into the cisterna magna, SPION-containing cells were found outside of the CNS and, particularly, in the cervical lymph node. This was observed in SIV-infected monkeys an in uninfected control monkeys. This is the first study to demonstrate that brain macrophages can migrate from the CNS into the periphery, thus creating the opportunity to seed tissues outside of the brain with viruses that have adapted to CNS replication.

All cited abstracts appear in the CROI 2016 Abstracts eBook, available online at www.CROIconference.org.

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

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