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Viral latency and potential eradication of HIV-1

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Abstract

Although HAART can suppress plasma viral loads to undetectable levels, individuals infected with HIV-1 harbor latent reservoirs of integrated proviruses that re-emerge upon the cessation of drug treatment. The 2012 Keystone Symposium on Frontiers in HIV Pathogenesis, Therapy and Eradication highlighted the current understanding of latent infection and new methods to activate and target these reservoirs for eradication. This report focuses on a select few aspects of the discussion, including the extent that ongoing replication might contribute to the persistent viral reservoir, recent advances in activating the expression of latent proviruses, progress in developing effective animal models and potential avenues to eradicate the cells that constitute the latent reservoir.

Keywords

HAART; HIV/AIDS; HIV-1 activation; HIV-1 replication; viral latency

Since the advent of AIDS three decades ago, great strides have been made in developing antiviral regimens capable of halting HIV-1 replication. Indeed, as described by Robert F Siliciano (Johns Hopkins School of Medicine, MD, USA), many of the multicomponent HAART regimens exhibit far greater potency than the estimated 6-$\log_{10}$ inhibitory potential necessary to suppress viral loads to undetectable levels [1]. Unfortunately, despite the effectiveness of HAART, it is incapable of virus eradication, as individuals removed from treatment exhibit a rebound in viral replication [2], seeded by the virus that persists within latent reservoirs [3]. Thus, recent focus has shifted to characterizing and eradicating these sources of latent viruses. It is important to note this is a lofty goal facing numerous hurdles, including identification of latent reservoirs, how to specifically activate their expression without rampant activation of the host immune response and how to eliminate the virus-expressing cells from the system once they are activated.

HIV-1 replication during suppressive HAART

In addition to obvious clinical benefits, the advent of HAART provided the means to measure the half-lives of plasma virus and infected cells due to the cessation of systemic

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virus replication [4]. Careful analysis has revealed four distinguishable slopes in the decay of plasma viremia, representing half-lives of corresponding infected cells of approximately 1–2 days, 2 weeks, 39 weeks and infinite [5,6]. Whereas activated $CD4^+$ T cells die on average 1.5 days postinfection [7], the identities of the cells that yield longer half-lives are largely undetermined. A popular candidate for the long-lived virus reservoir is quiescent $CD4^+$ T cells, which can be found in small numbers throughout the body [8]. This population might form when infected, activated $CD4^+$ cells return to the resting state as part of the memory response [9]. Alternatively, quiescent T cells, which can be infected at low efficiency *ex vivo*, might be directly infected *in vivo*, and thereby contribute to the latent reservoir [10]. Regardless, a prevailing view posits that long-lived cells are largely dormant, producing a small number of virus particles that are essentially noninfectious in the presence of HAART. Another view contends that some contribution of persistent infection is provided by low-level replication in sites hidden from HAART and immune surveillance. In previous work, Mario Stevenson (University of Miami Miller School of Medicine, FL, USA) and collaborators at the IrsiCaixa Institute in Barcelona, Spain, found that patients undergoing HAART intensification upon addition of the integrase inhibitor raltegravir exhibited increased levels of unintegrated, episomal HIV-1 DNA, suggestive of *de novo* infection [11]. Stevenson expanded upon this work by showing that certain tissues, such as lymph nodes and gut-associated lymphatic tissue, harbor increased levels of episomal viral DNA, suggesting that these tissues are potential sites of *de novo* replication. In addition, he reiterated that poor drug penetration in various tissues, such as tenofovir and atazanavir in lymph nodes, could underlie persistent replication. A key question related to the utility of using episomal viral DNA as a marker for new infection events centers on the stability of these unintegrated DNA species *in vivo*.

If there is smoldering replication in the presence of HAART, one would expect detectable viral sequence evolution over time, but work presented by Sarah Palmer (Karolinska Institute, Stockholm, Sweden) provided evidence against such an accumulation of genetic diversity. By performing single-genome sequencing from plasma, blood, bone marrow and gut-associated lymphatic tissue from patients on suppressive HAART, her group observed essentially no evidence for change over approximately 12 years of treatment. Furthermore, viral sequences in different tissues displayed similar genetic diversity, arguing against viral compartmentalization. Interestingly, one patient was found to harbor a provirus encoding a major inactivating deletion, which was probably clonally propagated by a memory T cell within the blood and bone marrow. Thus, any ongoing viral replication must be so little, or localized, so as to have escaped detection under these experimental conditions. Although technically difficult, additional studies must continue to probe various tissues, including lymph nodes, to fully characterize the dynamics of latency.

Activating & eradicating latent proviruses

Although quiescent $CD4^+$ T cells are currently the best-understood latent reservoir, the molecular mechanisms responsible for establishing proviral latency within these cells are only partially understood. Silencing of the integrated viral promoter via histone deacetylation would seem to play a major role [12]. Accordingly, the most tractable approach proposed to reactivate HIV-1 expression is inhibition of histone deacetylase (HDAC) activities, as illustrated *ex vivo* by drugs such as suberoylanilide hydroxamic acid (SAHA) [13]. Taking the drug into the clinic, David Margolis (University of North Carolina, NC, USA) showed significant increases in viral RNA within resting $CD4^+$ T cells upon SAHA application without corresponding change in plasma viremia. Kathryn Miller-Jensen (Yale University, CT, USA) investigated non-uniform reactivation by determining how local context of chromatin modulators, such as HDACs, DNA methylases and transcription factors such as NF-κB-RelA/p65, may affect the induction threshold of a silent HIV-1

promoter [14]. She also showed that HDAC inhibitors lower the threshold for induction, resulting in synergistic interactions with TNF-α. Still, a lingering concern is the safety of potentially activating a broad spectrum of cell types within the body. This may be partially alleviated in the future, as Daria Hazuda (Merck Research Laboratories, NJ, USA) described that SAHA derivatives that had lost the targeting of HDAC3, which is enriched in myeloid and T cells, also lost the ability to reactivate expression from resting $CD4^+$ T cells. Further testing of the sufficiency of HDAC3 inhibition for reactivation, as well as the continued development of more selective and potent compounds, will probably translate to greater tolerability and efficacy *in vivo*.

Animal models

However strong the insight gained through *ex vivo* HIV-1 replication models, extensive characterization of the latent reservoir and its potential pharmacological purging will require studying the virus in the context of systemic infection. J Victor Garcia (University of North Carolina) showed that humanized bone marrow/liver/thymus mice, which develop a fully functional human immune system, could be infected through rectal, oral, vaginal or intravenous inoculation. Viremia, which is fully suppressed by HAART [15], importantly rebounded following the discontinuation of treatment. Persistent low-level infection in lymphoid tissues and latent infection in resting T cells at frequencies similar to those seen in humans were also observed, representing a viable small animal model for studying viral latency and eradication [16]. Binhua Ling (Tulane National Primate Research Center, LA, USA) argued that SIV infection of Chinese rhesus macaques provides an ideal model for latency in long-term nonprogressing patients, as a higher rate of natural control is observed relative to that seen in the more commonly used Indian rhesus, and HAART suppressed plasma viremia with similar kinetics to that seen in humans. Furthermore, in the Chinese rhesus system, $CD4^+$, $CD8^+$ and memory T-cell populations in peripheral or lymphoid tissues behaved similarly to those in HIV-1-infected patients [17]. Zandrea Ambrose (University of Pittsburgh School of Medicine, PA, USA) described ongoing experiments testing viral latency in pigtailed macaques infected with SIV_{mne} encoding the HIV-1 reverse transcriptase enzyme, enabling them to include non-nucleoside reverse transcriptase inhibitors in HAART regimens. She obtained viral suppression in the majority of animals without detectable sequence evolution, indicating a lack of ongoing replication [18]. This model also recapitulated latent viral reservoirs in lymphoid tissues and the gut, the sizes of which correlated with plasma viremia levels at 1 week after infection, suggesting that they are seeded very early. This and similar models will enable her and others to probe viral reactivation following the administration of SAHA and other agents.

Enhancing the immune system to eradicate HIV-1

Recent results have indicated that activation of viral expression within latently infected $CD4^+$ cells using SAHA *ex vivo* is insufficient to kill cells via autologous T cells [19]. Thus, eradication will probably require boosting the immune response beyond that seen in most patients. Results garnered through studies of elite controllers of HIV-1 infection are inherently instructive. Bruce Walker (Ragon Institute, MA, USA) analyzed factors that cause some individuals harboring the protective *HLA-B*27* MHC allele to become elite controllers of HIV-1 infection. He found their immune reactions to be quantitatively similar to those of progressors, but qualitatively different through preferential cytotoxic T lymphocyte targeting of the immunodominant KK10 Gag epitope, with associations to distinct TCR-β clonotypes. This resulted in enhanced cytolytic activity and inhibition of viruses *ex vivo*, reflecting the characteristics necessary to invoke a highly effective cytotoxic T lymphocyte response. In a similar vein, Peter Kwong (National Institute of Allergy and Infectious Diseases, MD, USA) described a region on the viral surface envelope

glycoprotein 120 bound by broadly neutralizing antibodies that mimic the CD4 receptor and undergo extensive heavy-chain maturation [20]. He also reported atomic-resolution structures of the elusive V1/V2 variable loops in complex with a broadly neutralizing antibody that recognizes N-linked glycan. Although targeted vaccination to specific epitopes and structure-based vaccine design are inherently challenging, these studies reveal templates for potential prophylactic or therapeutic immunization.

Conclusion

HIV-infected individuals harbor multiple latent pools capable of reseeding viral replication upon HAART withdrawal. Long-lived quiescent $CD4^+$ T cells are a known reservoir for latent proviruses, but there is still debate as to whether viral latency is maintained solely through the slow decay of these and other long-lived cells, or by the addition of compartmentalized low-level replication. Direct experimentation on animal models, in combination with careful measurements collected from suppressed patients, provide a complement of approaches to test the efficacies of HDAC inhibitors, as well as other epigenetic activators, to reactivate latent gene expression. Still, selective targeting and eradication of the viral reservoir will require further development of therapeutic tools to enhance targeted immune responses in patients.

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