JCI The Journal of Clinical Investigation

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J Clin Invest. 2016;126(2):409-414. https://doi.org/10.1172/JCI86047.

Review Series

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Recent developments in the effort to cure HIV infection: going beyond N = 1

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Combination antiretroviral therapy (ART) can suppress plasma HIV to undetectable levels, allowing HIV-infected individuals who are treated early a nearly normal life span. Despite the clear ability of ART to prevent morbidity and mortality, it is not curative. Even in individuals who have full suppression of viral replication on ART, there are resting memory CD4$^+$ T cells that harbor stably integrated HIV genomes, which are capable of producing infectious virus upon T cell activation. This latent viral reservoir is considered the primary obstacle to the development of an HIV cure, and recent efforts in multiple areas of HIV research have been brought to bear on the development of strategies to eradicate or develop a functional cure for HIV. Reviews in this series detail progress in our understanding of the molecular and cellular mechanisms of viral latency, efforts to accurately assess the size and composition of the latent reservoir, the characterization and development of HIV-targeted broadly neutralizing antibodies and cytolytic T lymphocytes, and animal models for the study HIV latency and therapeutic strategies.

The past few years have seen a remarkable process of coalescence in which several distinct areas of AIDS research have been brought to bear on the search for a cure. The development of effective combination antiretroviral therapy (ART) in the mid-1990s initially raised hopes for a cure because plasma levels of HIV fell quickly to undetectable levels in treated patients (1–3). However, simultaneous studies demonstrated the presence in infected individuals of resting memory CD4$^+$ T cells harboring stably integrated viral genomes that did not produce infectious virus while the cells were in a resting state but could do so following T cell activation (4, 5). With the subsequent demonstration that this latent reservoir could persist indefinitely, even in the setting of optimal ART (6–11), hopes for a cure faded rapidly. In fact, cure became an almost taboo subject because of concerns that discussing cure would raise unrealistic expectations in infected individuals that this reservoir could ever be eliminated. In the meantime, steady improvements were made in the tolerability and convenience of ART regimens, such that infected individuals can now maintain indefinite suppression of viral replication to clinically undetectable levels on single-pill, once-daily regimens that have few side effects (12). Recent studies suggest that infected individuals starting treatment early with modern regimens have near-normal life expectancies (13–16).

The success of ART is one reason that there has been renewed interest in HIV cure. In addition, recent studies, beginning with the pioneering work of David Margolis and colleagues, have indicated that the latent reservoir can be perturbed in vivo with latency-reversing agents (LRAs) (17, 18). Importantly, the revival of optimism for HIV cure was further stimulated by the remarkable case of Timothy Brown, a courageous patient with HIV infection who received a hematopoietic stem cell transplant (HSCT) for leukemia from a carefully selected donor whose cells were resistant to HIV infection (19, 20). To date, Mr. Brown is the one and only person considered to be cured. His case gave rise to renewed hope that the problems posed by the latent reservoir could be overcome. In this issue of the *JCI*, a distinguished group of experts review exciting recent developments in the HIV cure field and describe how research discoveries in the areas of HIV pathogenesis, vaccines, and treatment are all now contributing to the search for a cure.

The latent reservoir as a barrier to cure

Although other reservoirs for HIV may exist, the latent reservoir in resting CD4$^+$ T cells is considered to be the major barrier to curing HIV infection. In everyone with HIV infection, regardless of how long they have been on suppressive ART, replication-competent virus can be isolated from resting CD4$^+$ T cells simply by activating the cells in vitro (6–10). T cell activation serves to reverse the state of latency that is observed in resting CD4$^+$ T cells, allowing the outgrowth of virus (4, 5). This concept underlies the quantitative viral outgrowth assay (QVOA) that is considered to be the "gold-standard" method for measuring the latent reservoir. The important problem of measuring the latent reservoir is discussed in detail by Marta Massanella and Douglas Richman in this issue (21). Longitudinal measurements by the QVOA in patients on suppressive ART established that the half-life of the pool of latently infected resting CD4$^+$ T cells is extremely long (3.7 years), thus requiring over 70 years of treatment to eradicate a pool of 10^6 cells (9, 10). This estimate was based on studies published in 1999 and 2003, when ART regimens still had considerable problems with side effects. Recently, Margolis and colleagues published a new longitudinal analysis of the half-life of the latent reservoir, as measured by the QVOA (22). Although many of the patients in this

Conflict of interest: The authors have declared that no conflict of interest exists.
Reference information: *J Clin Invest*. 2016;126(2):409–414. doi:10.1172/JCI86047.

study were on newer, less toxic regimens, the observed half-life was essentially the same (3.6 years). This means that the improvements in ART over the last several years have not affected the fundamental problem of HIV persistence in latently infected cells. The persistence of replication-competent HIV on a time scale of years in the setting of optimal ART has not been demonstrated for any other cell population, and thus the latent reservoir in resting $CD4^+$ T cells is a major focus of HIV cure research.

The question of why HIV establishes a state of latent infection is of considerable interest. Latency is a reversibly nonproductive state of infection of individual cells. For some viruses, especially those of the herpes virus family, latency is an important mechanism for immune evasion (23). It is not so clear that latency serves this function for HIV. Pioneering studies by Jeff Lifson and colleagues established that HIV replicates actively throughout the course of the infection in untreated individuals (24). The rapid evolution of escape mutants provides the principal mechanism by which the virus avoids elimination by antibody and cytolytic T lymphocyte (CTL) responses (25–30). Intriguing recent theoretical studies by Leor Weinberger suggest that latency is a "hard-wired" feature of the regulation of HIV gene expression that evolved as a "bet-hedging strategy" to allow the virus to be successfully transmitted across mucosal barriers, with subsequent reactivation once the initially infected cells reach a tissue site that is more favorable for viral replication (31, 32). If this hypothesis is correct, the form of latency involved is likely different from the one that allows persistence of the virus during ART. As is discussed below, the latter form of latency allows infected cells to persist for very long periods of time (months to years) without reactivation. In contrast, a long delay between mucosal exposure and systemic viral replication has never been reported.

The simplest explanation for the existence of the latent reservoir is that HIV latency is a consequence of viral tropism for activated $CD4^+$ T cells (4). T cell activation results in a gradual upregulation of the CCR5 coreceptor that is essential for entry of the commonly transmitted forms of HIV (33). Activation also increases deoxynucleoside triphosphate (dNTP) pools required for reverse transcription and releases sequestered forms of the host transcription factors NF-κB, nuclear factor of activated T cells (NFAT), and positive transcription elongation factor-b (PTEFb), all of which play an important role in HIV gene expression (as reviewed by Daniele Cary, Koh Fujinaga, and B. Matija Peterlin in this issue; ref. 34). Infection of activated $CD4^+$ T cells results in rapid reverse transcription, integration, viral gene expression, and virus production, followed generally by the death of the cells, usually in one to two days (35, 36). In contrast, infection of resting $CD4^+$ T cells is hindered by the absence of CCR5, by low levels of dNTPs maintained by the dNTP triphosphohydrolase SAMHD1 (37, 38), and by a recently described cell death pathway that is triggered by innate immune recognition of reverse transcription intermediates (39). While infection of both activated and resting $CD4^+$ T cells results in cell death, infection of activated $CD4^+$ T cells that are transitioning back to a resting state, while the cells are still permissive for reverse transcription and integration of the viral genome but not for high-level viral gene expression, may allow the establishment of a stable state of latent infection in resting $CD4^+$ T cells (4). This is a rare event, consistent with the extremely low frequency of latently infected cells in vivo ($1:10^6$). Viewed in this light, HIV latency is an unfortunate accident of viral tropism. Whether this or other explanations for the origins of HIV latency are correct, there is no doubt that latency is established in all infected individuals and that it serves as a formidable barrier to HIV cure.

A cure and some "near cure" cases

The single cure of HIV infection mentioned above involved a HSCT from an HLA-matched donor selected to be homozygous for a common 32–base pair deletion in the *CCR5* gene (19). As is reviewed by Daniel Kuritzkes in this issue (40), elimination of the latent reservoir in this patient likely involved the conditioning regimen given prior to transplant as well as the graft-versus-host reactions that occurred following transplant. Together, these factors resulted in complete or near-complete replacement of the host immune system (including latently infected cells) with HIV-resistant donor cells. Even if a small number of infected cells remained in this patient, any virus released would not be able to infect the HIV-resistant, donor-derived cells that now constitute his immune system. This remarkable case has proven that cure is possible.

Even more instructive were two subsequent cases in which HIV-infected individuals with recurrent lymphomas were given HSCTs from donors who were wild type at the *CCR5* locus (41, 42). ART was continued throughout the transplant period and for several years thereafter to protect donor cells from infection. ART was then discontinued, and, in both cases, the plasma virus levels remained below the limit of detection for several months. Normally there is an exponential increase in viremia at approximately two weeks after treatment interruption (43, 44). Both patients eventually experienced a sudden and dramatic rebound in viremia (at 3 and 8 months after ART interruption), presumably as the result of reactivation of one or more residual latently infected cells. A similar delay in viral rebound was observed in the "Mississippi baby," an infant born to an infected mother who had no prenatal care (45). High levels of HIV were detected in the baby shortly after birth, and an aggressive ART regimen was started immediately. Viremia fell rapidly to below the limit of detection and remained undetectable even after ART was interrupted (against medical advice) at approximately 15 months. Initially, there was hope that early ART had prevented the establishment of the latent reservoir, since the reservoir resides in memory $CD4^+$ T cells that are largely generated by exposure to antigen in postnatal life. However, there was again a sudden and dramatic rebound in viremia over 2 years after interruption of ART. Interestingly, all three cases were characterized by a lack of adaptive immune responses to HIV, either as a result of the transplant process or early treatment. In the absence of immune responses and ART, viral replication is exponential, with an R_0 of 20 (20 new infected cells for each infected cell) (46). Therefore, the prolonged periods of aviremia in these patients can only be explained by the persistence of HIV in a latent, nonreplicating form. These cases provide in vivo proof for the concept of HIV latency and demonstrate the difficulty of the problem we face in trying to cure the infection.

Mechanisms of persistence

The molecular mechanisms by which latency is established and maintained have been a subject of great interest and are reviewed here by Cary et al. (34). As is described in this elegant Review, there

are at least seven distinct mechanisms that could contribute to HIV latency. Some are clearly related to the activation state of the host cells and the fact that the transcriptional environment in resting $CD4^+$ T cells is nonpermissive for HIV gene expression (47, 48). Another mechanism is related to the nature of HIV integration sites in the human genome. Following reverse transcription, the double-stranded DNA form of the HIV genome is stably integrated into host cell DNA, typically within the introns of actively expressed host genes (49, 50). This can lead to a phenomenon of transcription interference, in which transcription complexes initiated at the upstream host promoter interfere with HIV transcription (51).

Interestingly, the site of HIV integration may have another effect on viral persistence. Recent studies from the laboratories of Frank Maldarelli and Lisa Frenkel have used integration site analysis to identify expanded clones of cells carrying HIV proviruses (52, 53). The identification of multiple infected cells with exactly the same integration site in the 3 billion–base pair human genome can only be explained by the proliferation of cells after infection. This raises the disturbing possibility that latently infected cells could undergo continuous clonal expansion, hampering efforts to eliminate the reservoir. Interestingly, in some of the expanded cellular clones, the provirus is integrated in genes associated with cell survival and/or proliferation, leading to the hypothesis that the presence of the provirus in these locations alters expression of the relevant host genes in a way that promotes proliferation of the infected cells.

A major issue is whether the expanded cellular clones detected by integration site analysis harbor replication-competent virus. Detailed analysis of proviruses in resting $CD4^+$ T cells from patients on ART has shown that the vast majority have inactivating defects in the form of hypermutation induced by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) or large internal deletions arising during reverse transcription (54). Cohn and colleagues examined 75 expanded cellular clones identified through integration site analysis and showed that all carried defective proviruses (55). Nevertheless, it remains possible that a small fraction of these expanded cellular clones harbor replication-competent HIV. Studies of the trace levels of free virus in plasma of patients on ART have previously demonstrated the dominance of clonal populations of virus, presumably derived from expanded cellular clones capable of producing virus particles (56). This issue and the overall concept of clonal expansion are discussed in detail by Frank Maldarelli in this issue (57). How the existence of expanded cellular clones alters the approach to elimination of the reservoir is a currently a subject of great interest.

"Shock and kill"

The most widely discussed approach for eliminating the reservoir is the "shock and kill" approach, in which small-molecule LRAs are used to induce HIV gene expression in the hopes that the infected cells will then die as a result of viral cytopathic effects and/or natural or induced HIV-specific immune responses (17, 58–66). This would be done in patients on ART, and it is generally assumed that reversal of latency will not result in new infection events, given the remarkable efficacy of antiretroviral drugs (67, 68). Numerous LRAs have been identified in studies with transformed cell lines carrying latent HIV proviruses and various primary T cell models of latency (reviewed in refs. 69, 70). T cell activation was used to reverse latency in the original studies defining the latent reservoir (4, 5), but this activation is associated with substantial toxicities that preclude the use of this approach therapeutically (71, 72). Nevertheless, LRA activity should be compared to a positive control in which global T cell activation is induced by mitogens or a combination of anti-CD3 and anti-CD28 antibodies. For some of these LRAs, notably the PKC agonists, there are obvious mechanistic explanations for their activity related to effects on signaling pathways involved in T cell activation. For other LRAs, including the histone deacetylase (HDAC) inhibitors, the mechanisms remain controversial (65, 73). One of the major problems in the search for effective LRAs is that, despite impressive activity in various in vitro models, most LRAs, including the HDAC inhibitors, have weak activity in ex vivo studies using resting $CD4^+$ T cells from patients on ART (74). Fortunately, some combinations of LRAs are now beginning to show levels of latency reversal comparable to global T cell activation (75, 76). In clinical trials, no reduction in the reservoir has yet been demonstrated, but there is evidence for increases in cell-associated HIV RNA and for slight transient increases in plasma HIV RNA with certain HDAC inhibitors (17, 18). Together, these findings suggest that it will be possible to reverse HIV latency in vivo.

Reversal of latency will not reduce the reservoir unless the infected cells die as a result. Studies in primary cell models of HIV latency suggest that infected cells may not die as a result of viral cytopathic effects following LRA treatment (77). In elegant humanized mouse models of HIV infection, the elimination of infected cells has been shown to be dependent upon additional interventions designed to kill infected cells (48, 78). (Rapid progress in the use of humanized mice to study HIV eradication strategies is discussed by J. Victor Garcia in this issue; ref. 79.) Following latency reversal, infected cells will likely express viral antigens, but it is not clear that they will be eliminated by host CTL responses. As is discussed in a comprehensive Review by Jones and Walker in this issue (80), these responses wane in patients on ART due to the absence of antigen, and there are lingering effects of the immune exhaustion seen in untreated HIV infection (81, 82). In addition, recent studies have demonstrated that unless ART is started very early in the course of HIV infection, the latent reservoir is composed almost entirely of viruses with escape mutations in dominant CTL epitopes (30). Jones and Walker consider differences in features of the CTL response needed for control of active replication versus the clearance of latently infected cells and discuss new results on the targeting of epitopes, which, if mutated, lead to structural instability of the relevant viral proteins (80). Other recently identified problems with the CTL response include the negative effects of some LRAs on CTL function (84) and the possibility that infected cells may persist in anatomical sites that are inaccessible to CTLs, including the germinal centers of the lymph nodes, in which viral replication can occur in $CD4^+$ T follicular helper cells (85, 86). Despite these problems, there is hope that an appropriately primed $CD8^+$ T cell response may facilitate clearance of infected cells following latency reversal.

Recently, an unusual form of T cell immunity has been show to play a role in the clearance of infected cells in an important animal model of HIV infection, namely SIV infection of rhesus

macaques. A CMV-based vaccine carrying SIV antigens provided durable protection against challenge with a pathogenic strain of SIV in 50% of vaccinated animals (87–89). In these animals, there was an initial burst of SIV replication that was rapidly controlled. The animals went on to completely clear the infection, as demonstrated by the transfer of cells to naive animals. The clearance of SIV-infected cells was mediated by $CD8^+$ T cells, but the specificity of the cells was atypical. A broad array of SIV peptides was recognized by these cells in the context of class II and nonclassical class I MHC molecules. Whether similar noncanonical responses can be induced in humans with CMV vectors and whether they will be useful in HIV eradication strategies remain important questions.

Another approach to the elimination of infected cells involves the use of antibodies directed at the HIV envelope protein, which should be expressed on the cell surface following reversal of latency. The last few years have seen extraordinary progress in our understanding of the structure of the envelope protein and of the antibody response that it induces. In particular, there has been interest in broadly neutralizing antibodies (bNAbs) that can recognize a very wide range of HIV isolates. This work is reviewed here by Ariel Halper-Stromberg and Michel Nussenzweig (90). Nussenzweig's group developed methods for the cloning of these antibodies. Many of them have unusual structural features that are the result of a lengthy process of coevolution of the virus and the antibody response in infected individuals (26, 27). While these antibodies may be difficult to induce by vaccination, there is hope that passive infusion of bNAbs could contribute to the killing of latently infected cells if done in conjunction with LRA treatment. As is reviewed by Halper-Stromberg and Nussenzweig, this killing may involve the Fc receptor–dependent engagement of NK cells (90).

Summary

The renewed interest in HIV cure has brought together diverse lines of HIV research, including studies of HIV molecular biology, pathogenesis, and vaccine development, all of which may end up contributing to a cure. Although infected individuals with access to ART can now expect much better clinical outcomes than those in the pre–ART era, they still face a lifetime of treatment. The global burden of treating every infected individual for life with combinations of expensive antiretroviral drugs is a strong motivation for finding simple, scalable, curative regimens that can reduce the viral reservoir and allow prolonged ART-free remissions.

Acknowledgments

This work was supported by the Martin Delaney Collaboratory of AIDS Researchers for Eradication and Delaney AIDS Research Enterprise (NIH grants AI096113 and 1U19AI096109), by an amfAR Research Consortium on HIV Eradication collaborative research grant from the Foundation for AIDS Research (amfAR 108165-50-RGRL), by the Johns Hopkins Center for AIDS Research (P30AI094189), by NIH grant 43222, by the Howard Hughes Medical Institute, and by the Bill and Melinda Gates Foundation.

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