The Remarkable Stability of the Latent Reservoir for HIV-1 in Resting Memory CD4⁺ T Cells

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(See the major article by Crooks et al on pages 1361–5.)

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The modern era of antiretroviral therapy (ART) for human immunodeficiency virus type 1 (HIV-1) infection began in the mid-1990s with the introduction of 2 new classes of antiretroviral drugs, the protease inhibitors (PIs) and the nonnucleoside reverse-transcriptase inhibitors. Combinations consisting of 1 of these drugs along with 2 nucleoside analogue reverse-transcriptase inhibitors rapidly reduced plasma HIV-1 RNA levels to below the limit of detection of clinical assays [1, 2], leading to predictions that continued treatment for 2–3 years could cure the infection [3]. Although it did not prove curative, combination ART became the mainstay of HIV treatment, allowing durable control of viral replication and reversal or prevention of immunodeficiency [4].

A major reason why ART did not prove curative is the persistence of a latent form of the virus in a small population of resting memory CD4⁺ T cells [5, 6]. In these cells, the viral genome is stably integrated into host cell DNA, but viral genes are not expressed at significant levels in part because of the absence of key host transcription factors that are recruited to the HIV promoter only after T-cell activation. The latent reservoir for HIV-1 was originally demonstrated using an assay in which resting cells from patients are activated to reverse latency [6]. Viruses released from individual latently infected cells are expanded in culture. This viral outgrowth assay (VOA) was used to demonstrate the remarkable stability of the latent reservoir [7–9]. The half-life of this pool of cells was shown to be 44 months. At this rate of decay, >70 years would be required for a pool of just 10⁶ cells to decay completely [8, 9].

Initial studies of the decay of the latent reservoir were completed in 2003 [9]. Since that time, remarkable advances in ART have taken place, including the introduction of new classes of antiretroviral drugs, such as integrase inhibitors, and the development of simplified regimens in which multiple antiretroviral drugs are combined into a single pill that can be taken once daily [4]. In this context, an extensive and careful study of latent reservoir decay by Crooks et al [10], reported in this issue of the Journal, is of particular interest. The authors have reexamined the stability of the latent reservoir using longitudinal VOAs in a series of 37 patients, some of whom have been receiving treatment for most of the modern ART era. Despite the long duration of treatment in some patients and the changes in ART, the authors found that the decay rate of the latent reservoir is almost exactly the same as that reported in 2003. The half-life measured by Crooks et al is 43 months [10].

The fact that the decay rate measured in the present study is no different from that measured more than a decade ago confirms that the stability of the latent reservoir is not determined by treatment regimens. As long as the regimen produces a complete or near-complete arrest of new infection events, the decay of the reservoir is determined by the biology of the resting memory T cells that harbor persistent HIV-1. Pharmacodynamic studies indicate that the nonnucleoside reverse-transcriptase inhibitors and PIs possess a remarkable potential to inhibit viral replication, a property that reflects an unexpected degree of cooperativity in their dose-response curves [11, 12]. At clinical concentrations, the best PIs can actually produce a 10 billion–fold inhibition of a single round of HIV-1 replication. Thus, even the early combination therapy regimens may have produced complete or near-complete inhibition of new infection events in drug-adherent patients. Subsequent improvements in ART have largely affected tolerability and convenience. Viewed in this light, the finding that the reservoir decay is constant is not surprising. The cures now being routinely achieved with direct-acting antiviral drugs...
against hepatitis C virus are another reflection of the remarkable ability of certain antiviral drugs to block viral replication. The big difference, of course, is that there is not a latent reservoir for hepatitis C virus. Finding ways to eliminate the latent reservoir for HIV-1 has become a major focus of HIV research.

The results reported by Crooks et al [10] highlight the remarkable stability of this reservoir. The stability can be explained by the fact that latent HIV-1 resides predominantly in memory CD4+ T cells [6]. Memory T-cell responses in humans decay very slowly, with a half-life of 8–15 years [13]. This reflects the long-term survival in individual memory T cells as well as the homeostatic proliferation process that preserves immunologic memory to specific pathogens at protective levels for life. Several studies provide evidence for the clonal expansion of populations of HIV-1–infected cells [14–17]. The trace amount of free virus found in the plasma of patients receiving suppressive ART seem to originate from stable reservoirs [18] and is frequently oligoclonal [14, 15], suggesting that this residual viremia may be produced by cells that have undergone clonal expansion after infection. More recently, 2 laboratories have provided direct evidence for clonal expansion of infected cells by demonstrating multiple cells with the same viral integration site [16, 17]. HIV-1 typically integrates into actively expressed genes throughout the genome [19, 20]; thus, each integration event is unique. The repeated detection of identical integration sites suggests that some infected cells can proliferate after infection. A caveat to this notion is that the integration site studies capture only the very ends of the HIV-1 genome. Some findings suggest that most proviruses in resting CD4+ T cells harbor large internal deletions and/or APOBEC3G-mediated hypermutation and are thus not competent for replication [21]. Whether clonal expansion plays a major role in the persistence of cells with replication-competent virus is still a matter of debate. However, regardless of the mechanism of persistence, the study by Crooks et al [10] confirms that the latent reservoir is sufficiently stable to preclude HIV-1 eradication without specific interventions to target the reservoir.

Since the initial report of the cure of a single patient by a hematopoietic stem cell transplant [22], there has been a dramatic increase in optimism that HIV-1 infection may be curable if the problem of the latent reservoir can be overcome. An important study by Archin et al [23] showed that the reservoir can be perturbed in vivo by a histone deacetylase inhibitor, and many trials of latency reversing agents are now beginning [24, 25].

One critical issue going forward is how to measure the reservoir in these eradication studies. There is no clinical assay for the latent reservoir. The VOA, which has long been regarded as the gold standard, is expensive and time consuming. Because of the low frequency of latently infected cells (mean frequency, <1/106 resting CD4+ T cells), large sample volumes are required, typically 180 mL of blood or, as described by Crooks et al [10], leukapheresis. The assay requires 2–3 weeks of tissue culture work in a biosafety level 3 facility. For these reasons, there has been great interest in alternatives to the VOA, particularly polymerase chain reaction (PCR)–based assays. However, a recent comparative study showed that PCR-based assays yield infected cell frequencies that are 2-fold and rarely differ by >6-fold. They suggest 6-fold as a credible threshold for an intervention-induced decline in the reservoir, although much larger declines may be necessary to produce a substantial delay in the time to viral rebound after discontinuation of ART [27].

Crooks et al [10] also clarify an important issue related to the decay of the reservoir in patients who start treatment early in the course of infection. Although findings of several studies suggest that early treatment may result in a smaller reservoir, a stable reservoir can be seeded within a matter of days after infection [28] and, in contrast to some previous reports, the authors found no difference in reservoir decay rates between patients starting treatment during acute versus chronic infection. Thus, although early treatment is preferred for many patients, it is unlikely to overcome the problem of the latent reservoir. It is likely that all infected individuals will require interventions directly targeting the reservoir to achieve a cure.

**Note**

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**References**


