Early Treatment and HIV-1 Reservoirs: A Stitch in Time?

Timothy J. Henrich1,2 and Rajesh T. Gandhi2,3

1Division of Infectious Diseases, Brigham and Women’s Hospital; 2Harvard Medical School, and 3Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts; Ragon Institute of MGH, MIT, Harvard

(See the major articles by Jain et al on pages 1202–11 and Yuki et al on pages 1212–20.)

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Several recent high-profile studies have highlighted the potential impact of antiretroviral therapy (ART) initiation during acute human immunodeficiency virus type 1 (HIV-1) infection on viral reservoirs and persistence. Exciting observations presented at this year’s “Conference on Retroviruses and Opportunistic Infections” [1] on a child in Mississippi that started ART within hours of birth to an infected mother suggested that immediate treatment prevented HIV-1 from establishing a foothold in the infant, leading to lack of viral rebound after ART cessation. Two additional studies suggested that initiating early ART in adults with acute HIV-1 infection might limit seeding of key viral reservoirs, including those found in specific types of memory CD4+ T cells [2–4]. Although the long-term significance of the observations in these provocative reports is not yet known, they have invigorated the field of HIV-1 curative strategies and persistence research. In this issue of The Journal of Infectious Diseases, 2 articles add to the growing discussion regarding the impact of early ART on reservoir size and T-cell activation, and help elucidate the composition of the HIV-1 reservoir in CD4+ T-cell subsets in blood and gastrointestinal tissue.

Early ART and T-cell Activation

Patients treated during the chronic phase of HIV-1 infection have abnormal levels of immune activation as well as persistence of virus in memory CD4+ T cells, even after years of ART. To evaluate whether treatment during acute infection impacts immune activation and virus reservoirs, Jain and colleagues [5] compared T-cell activation and HIV-1 nucleic acid levels in patients who started ART <6 months from the time of infection (early ART) to those who started therapy ≥2 years after infection (later ART). Participants had suppressed virus for a minimum of 2 years prior to the final study time point. Although ART led to declines in T-cell activation in both groups, the early ART group was found to have significantly lower levels of activated CD4+ and CD8+ T cells when compared with the later ART group. It was unclear, however, whether activation levels immediately before ART initiation were similar between the early and later ART groups, and intergroup differences could have affected these observations.

Despite the clear-cut effect of ART on reducing immune activation, CD8+ T-cell activation levels remained higher in the early therapy group than in HIV-seronegative controls; another recent study showed similar findings [6]. Higher levels of persistent T-cell activation may, in part, be related to damage to the immune system that occurs prior to ART initiation. In the current study and a previous one from the AIDS Clinical Trials Group [7], lower pre-ART CD4+ T-cell count was associated with persistently elevated CD4+ T-cell activation in patients on ART. One interpretation of these studies is that early ART initiation limits, but does not completely abrogate, damage to the immune system. In this regard, the impact of early ART on other measures of immune activation and inflammation, such as soluble biomarkers, is an important area of investigation. For example, monocyte-macrophage activation markers, which are linked to arterial inflammation and atherosclerosis [8, 9], normalize in patients treated with early ART [6, 10].

Early ART and HIV-1 Reservoirs

Among the possible benefits of diminishing T-cell activation is a reduction in viral reservoirs, as higher proviral DNA and cell-associated RNA levels are correlated with increased frequencies of activated CD4+ and CD8+ T cells [11]. (An alternative explanation is that higher HIV-1
levels are driving T-cell activation; only interventional studies will be able to sort out the direction of causality.) Jain et al [5] observed that the early ART group had lower levels of HIV-1 DNA and cell-associated RNA at the last available treatment time point than those who initiated ART later. However, it is unclear whether HIV-1 DNA and RNA levels were similar between the early and later treatment groups just prior to ART initiation; it is possible that pretreatment DNA levels were higher in the later-ART group. Furthermore, because this was not a randomized study, there may have been differences between the early and later ART groups that influenced the on-treatment reservoir size independent of ART timing. Nonetheless, Jain et al [5] found significant associations with timing of ART and cellular reservoir size in covariate-adjusted longitudinal mixed-effects regression models. Moreover, the results are consistent with those of other studies finding that patients treated during acute infection have smaller HIV-1 reservoirs, at least by some measures, than those treated during chronic infection [12–14].

Because establishment of the HIV-1 reservoir occurs soon after primary infection [15, 16], how quickly therapy is started may make a substantial difference. For example, in a study from Thailand [2, 4], levels of cell-associated HIV-1 DNA were found to be significantly lower during the earliest phase of acute infection when compared with later stages of primary infection. In patients who initiated therapy prior to seroconversion, there was a positive correlation between extent of pretreatment viremia and the frequency of latently infected resting CD4+ T cells [15]. Similarly, Jain et al [5] found that pre-ART cumulative viremia predicted on-ART cellular RNA levels. The rapid seeding of reservoirs soon after HIV-1 acquisition means that very early ART will be required to limit widespread establishment of latent infection in susceptible cells.

Moreover, whether reducing HIV-1 reservoir size by initiation of extremely early therapy will prevent virologic rebound when treatment is stopped is an open question. In a recent French study [3], 14 patients who initiated ART in the early stages of acute infection had control of viremia after treatment interruption (posttreatment controllers). However, in a previous study [17], viral control after treatment interruption was not durable in a majority of patients treated during acute infection. The mechanism by which sustained viral control was achieved in the French posttreatment controllers is not yet certain.

**Memory CD4+ T-cell Subsets and the HIV-1 Reservoir**

One possible mechanism for post-treatment viral control is that early ART may impact the distribution of HIV-1 in different memory CD4+ T-cell subsets (Figure 1), which might affect viral persistence and rebound. In the peripheral blood of patients on ART, HIV-1 is found predominantly in memory CD4+ T cells rather than in naïve cells [12, 18, 19]. In those with high CD4+ cell counts, much of the HIV-1 reservoir is found in central memory CD4+ T cells (T\textsubscript{CM}); by contrast, in those with low CD4+ T-cell counts, infected transitional memory (T\textsubscript{TM}) and effector memory (T\textsubscript{EM}) CD4+ T cells contribute to a larger portion of the reservoir [12]. While T\textsubscript{CM} cells are intrinsically long-lived, the T\textsubscript{TM} viral reservoir appears to be replenished by homeostatic proliferation of latently infected cells [12]. Interestingly, in post-treatment controllers, the shorter-lived T\textsubscript{TM} subset appears to be the main contributor to the HIV-1 reservoir [3]. In addition, early ART initiation in the Thai study [4] led to restricted seeding of the T\textsubscript{CM} reservoir and very low or undetectable levels of integrated HIV-1 DNA.

If persistence of HIV-1 is more stable in T\textsubscript{CM} cells, then the skewing of the reservoir into shorter-lived T\textsubscript{TM} cells might explain the gradual decrease in the HIV-1 reservoir seen in posttreatment controllers [3]. As memory CD4+ T cells are present at lower levels in newborns [20], early treatment may have prevented HIV-1 from gaining a foothold in the previously described Mississippi child. Limited infection of T\textsubscript{CM} cells is also observed in patients with protective human leukocyte antigen alleles and spontaneous viral control [21]. Whether T\textsubscript{CM} can be protected from infection by other interventions or whether infected T\textsubscript{CM} can be eliminated through novel approaches are high-priority questions for the field. However, even if T\textsubscript{CM} infection can be limited, an even more daunting obstacle to HIV-1 eradication may be infection of stem cell memory lymphocytes (T\textsubscript{SCM}) [22], extremely long-lived cells with the capacity for self-renewal and homeostatic proliferation.

Although there have been important advances in our understanding of the distribution of HIV-1 in memory T-cell subsets, much of our knowledge has been limited to findings made in peripheral blood. There is a growing realization that we need more insight into the composition of the HIV-1 reservoir in tissues, such as the gut, which is the site of massive CD4+ T-cell depletion during acute infection [23, 24], and of viral persistence in patients on ART [25]. In this issue of the Journal, Yukl and colleagues [26] report a study of HIV-1 reservoirs in memory T-cell subsets from blood and gastrointestinal tissue in 8 HIV-infected patients on suppressive ART who underwent colonoscopy. Most of the patients had high CD4+ T-cell counts at the time of biopsy, although several had low nadirs in the past. As in a previous study by this group [25], HIV-1 DNA levels were found to be higher in the rectum and ileum than in the blood. In peripheral blood, the largest proportion of the HIV-1 DNA and cell-associated RNA was found in CCR7+ cells (which comprise naïve T-cells, T\textsubscript{CM}, and other memory cells). By contrast, in the ileum and rectum, the largest proportion of HIV-1 DNA and RNA was found in T\textsubscript{EM} cells. As expected, the T\textsubscript{CM} pool was the most prevalent memory cell type in the blood, but T\textsubscript{EM} constituted the main pool.
in the ileum and rectum. The relative frequencies of T-cell subsets roughly correlated with the total contributions of HIV-1 DNA and cell-associated RNA reservoirs in those subsets to the gut reservoir. HIV-1 DNA and RNA were also found in non-CD4+ leukocytes, particularly in the rectum and ileum, although it is unclear exactly which cell types constituted this population.

This important study shows that blood-based measurements of the HIV-1 reservoir do not always reflect events occurring in tissues and suggests that differences in blood and tissue cellular environments may significantly impact the size and distribution of the viral reservoir in patients on suppressive ART. For example, markers for lymphocyte activation are higher in the gut than in the blood, which may arise from microbial translocation and potentially lead to increased presence of infected T_{EM} subsets, as observed by Yukl et al [26, 27]. More studies are needed to understand how HIV-1 persists in tissues and, by extension, how best to target those reservoirs for elimination.

**What Is to Be Done?**

There is accumulating evidence for the benefits of treating patients during acute HIV-1 infection [28]; the finding that early ART is associated with lower T-cell activation supports this view and points to the need for studies of whether such therapy improves end-organ function and clinical endpoints. In addition, the impact of early ART on limiting HIV-1 reservoir size suggests that patients treated during acute infection might be an ideal population for future eradication studies. In addition to having smaller blood reservoirs, patients treated during acute infection have more limited virus diversity [29–31] and may have a smaller proportion of defective provirus than those treated later in infection. These characteristics may make latency-activating factors and therapeutic vaccination strategies more effective. Studies evaluating the effects of such interventions in patients treated during acute infection are a high priority for advancing our knowledge of HIV-1 reservoir size and function.

In addition, we need a more comprehensive understanding of what determines the distribution and persistence of HIV-1 in different memory T-cell subsets in blood and tissues, both in patients...
treated during chronic infection (as in the study by Yukl et al [26]) as well as in patients treated during acute infection. We also need investigations of whether interventions designed to purge latent reservoirs, such as histone deacetylase inhibitors, have differential effects on latent HIV-1 infection in various T-cell subsets. Finally, studies to test the exciting possibility that specific memory CD4 cell subsets play a critical role in the stability of the HIV-1 reservoir should be vigorously pursued and rigorously assessed.

The field of HIV-1 eradication research was jump-started 5 years ago with the report of the first cure [32], and has been accelerated in recent years by tantalizing studies suggesting that very early treatment may limit the size of HIV-1 reservoirs. Although the road ahead is likely to be full of twists and turns, what is certain is that pathogenesis-based investigations on the determinants and composition of the viral reservoir—as exemplified by these 2 studies—will improve the conception, implementation, and targeting of novel strategies designed to eradicate HIV-1 infection.

Notes

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