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

Timothy J. Henrich[1,2] and Rajesh T. Gandhi[2,3]

[1]Division of Infectious Diseases, Brigham and Women's Hospital; [2]Harvard Medical School, and [3]Division 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 Yukl et al on pages 1212–20.)

Keywords. HIV; antiretroviral therapy; HIV reservoirs; HIV persistence; gut-associated-lymphoid-tissue.

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

Received 16 May 2013; accepted 20 May 2013; electronically published 12 July 2013.

Correspondence: Rajesh T. Gandhi, MD, Division of Infectious Diseases, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114 (rgandhi@partners.org).

The Journal of Infectious Diseases 2013;208:1189–93

DOI: 10.1093/infdis/jit307

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.

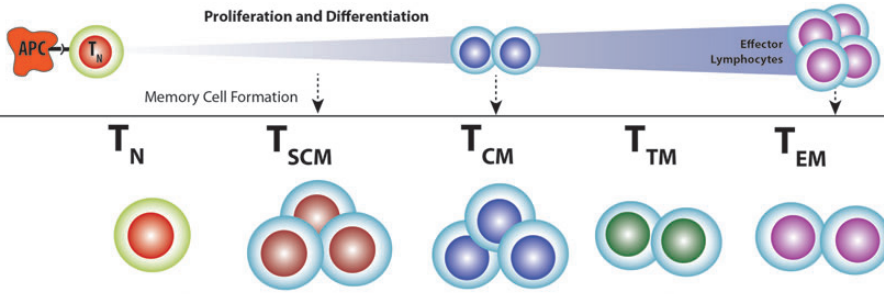
Moreover, whether reducing HIV-1 reservoir size by initiation of extremely early therapy will prevent virologic rebound when treatment is stopped is an open question. In a recent French study [3], 14 patients who initiated ART in the early stages of acute infection had control of viremia after treatment 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 naive 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_{CM}); by contrast, in those with low $CD4^+$ T-cell counts, infected transitional memory (T_{TM}) and effector memory (T_{EM}) $CD4^+$ T cells contribute to a larger portion of the reservoir [12]. While T_{CM} cells are intrinsically long-lived, the T_{TM} viral reservoir appears to be replenished by homeostatic proliferation of latently infected cells [12]. Interestingly, in posttreatment controllers, the shorter-lived T_{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_{CM} reservoir and very low or undetectable levels of integrated HIV-1 DNA.

If persistence of HIV-1 is more stable in T_{CM} cells, then the skewing of the reservoir into shorter-lived T_{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_{CM} cells is also observed in patients with protective human leukocyte antigen alleles and spontaneous viral control [21]. Whether T_{CM} can be protected from infection by other interventions or whether infected T_{CM} can be eliminated through novel approaches are high-priority questions for the field. However, even if T_{CM} infection can be limited, an even more daunting obstacle to HIV-1 eradication may be infection of stem cell memory lymphocytes (T_{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 naive T-cells, T_{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_{EM} cells. As expected, the T_{CM} pool was the most prevalent memory cell type in the blood, but T_{EM} constituted the main pool



	T_N	T_{SCM}	T_{CM}	T_{TM}	T_{EM}
CD45RA	+	+	-	-	-
CD45RO	-	-	+	+	+
CD27	+	+	+	+	-
CCR7 (lymph/gut homing)	+	+	+	-	-
Prevalence					
Peripheral Blood	+++	*	+++	++	++
Ileum\Rectum	+/-		+	++	+++
Contribution to HIV-1 DNA Reservoir					
Peripheral Blood (High $CD4^+$ T-cell count) [12]			+++	++	+
(Low $CD4^+$ T-cell count) [12]			++	+++	+(?)
Ileum			+**	+	+++
Rectum			+**	++	+++

Figure 1. The contributions of memory $CD4^+$ lymphocyte subsets in gut and peripheral blood to the HIV-1 reservoir are heterogeneous. During the course of a normal immune response, a fraction of antigen-activated $CD4^+$ T cells differentiate into memory cells. Memory $CD4^+$ T cells can be further divided into central memory T cells (T_{CM}), which have limited effector function and traffic to secondary lymphoid tissues, and effector memory T cells (T_{EM}), which actively express effector functions. T_{CM} may be induced to become T_{EM} after T-cell receptor triggering or in response to cytokines. Transitional memory (T_{TM}) T cells have characteristics intermediate between those of T_{CM} and T_{EM}. T_{CM} are the most prevalent memory subset in peripheral blood and the largest contributor to the DNA reservoir in blood in patients with higher $CD4^+$ T-cell counts (T_{TM} contribute significantly to the blood reservoir in patients with low $CD4^+$ counts). T_{EM} cells are most prevalent in the gut tissue and, as a result, are the predominate contributors to the HIV-1 DNA reservoir in the ileum and rectum. *Stem cell memory lymphocytes (T_{SCM}) [33], which self-renew and undergo homeostatic proliferation, have recently been shown to harbor HIV-1 and may play a role in viral persistence [22]. **Reservoirs were measured combining all $CCR7^+$ cells (naive and central memory subsets). Abbreviation: T_N, naive T cells.

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-cells 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 twist 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

Acknowledgments. We acknowledge Daniel Kauffman and Nicolas Chomont for their insights into this editorial.

Financial support. This work was supported by the National Institutes of Health (NIH) (R01 AI066992-04A1 to R.T.G.) and NIH G08LM008830-01 (to R.T.G.) and by grants to the AIDS Clinical Trials Group (NIH U01 AI 694722) and the Harvard University Center for AIDS Research (NIH 2P30 AI060354-06) (to R.T.G.). T.J.H. is supported by the NIH (1K23AI098480-01), the Foundation for AIDS Research (amfAR) (108466-52-RGRL), and the Harvard University Center for AIDS Research (NIH P30 AI060354).

Potential conflicts of interest. R.T.G. has received educational grant support from Janssen, Viiv, and Abbott. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed

References

1. Persaud D, Gay H, Ziemniak C, et al. Functional HIV cure after very early ART of an infected infant [abstract #48LB]. In: 20th Conference on Retroviruses Opportunistic Infections, Atlanta, GA, 3–6 March 2013.
2. Ananworanich J, Schuetz A, Vandergeeten C, et al. Impact of multi-targeted antiretroviral treatment on gut T cell depletion and HIV reservoir seeding during acute HIV infection. PLOS ONE **2012**; 7: e33948. doi:10.1371/journal.pone.0033948.
3. Saez-Cirion A, Bacchus C, Hocqueloux L, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLOS Pathog **2013**; 9:e1003211.
4. Ananworanich J, Vandergeeten C, Chomchey N, et al. Early ART intervention restricts the seeding of the HIV reservoir in long-lived central memory CD4 T cells [Abstract #47]. In: 20th Conference on Retroviruses and Opportunistic Infections. Atlanta, GA, 3–6 March 2013.
5. Jain V, Hartogensis W, Bacchetti P, et al. Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower t-cell activation and smaller HIV reservoir size. J Infect Dis **2013**; 208:1202–11.
6. Vinikoor MJ, Cope A, Gay CL, et al. Antiretroviral therapy initiated during acute HIV infection fails to prevent persistent T cell activation. J Acquir Immune Defic Syndr **2013**; 62:505–8.
7. Robbins GK, Spritzler JG, Chan ES, et al. Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 384. Clin Infect Dis **2009**; 48:350–61.
8. Pereyra F, Lo J, Triant VA, et al. Increased coronary atherosclerosis and immune activation in HIV-1 elite controllers. Aids **2012**; 26:2409–12.
9. Subramanian S, Tawakol A, Burdo TH, et al. Arterial inflammation in patients with HIV. JAMA **2012**; 308:379–86.
10. Burdo TH, Lentz MR, Autissier P, et al. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. J Infect Dis **2011**; 204:154–63.
11. Hatano H, Jain V, Hunt PW, et al. Cell-based measures of viral persistence are associated with immune activation and programmed cell death protein 1 (PD-1)-expressing $CD4^+$ T cells. J Infect Dis **2013**; 208:50–6.
12. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med **2009**; 15:893–U92.
13. Eriksson S, Graf EH, Dahl V, et al. Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. PLOS Pathog **2013**; 9:e1003174.
14. Strain MC, Little SJ, Daar ES, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. J Infect Dis **2005**; 191: 1410–8.
15. Archin NM, Vaidya NK, Kuruc JD, et al. Immediate antiviral therapy appears to restrict resting $CD4^+$ cell HIV-1 infection without accelerating the decay of latent infection. Proc Natl Acad Sci USA **2012**; 109:9523–8.
16. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc Natl Acad Sci USA **1998**; 95:8869–73.
17. Kaufmann DE, Lichterfeld M, Altfeld M, et al. Limited durability of viral control following treated acute HIV infection. PLOS Med **2004**; 1:e36.
18. Brenchley JM, Hill BJ, Ambrozak DR, et al. T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: Implications for HIV pathogenesis. J Virol **2004**; 78:1160–8.
19. Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature **1997**; 387:183–8.
20. Shearer WT, Rosenblatt HM, Gelman RS, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol **2003**; 112:973–80.
21. Descours B, Avettand-Fenoel V, Blanc C, et al. Immune responses driven by protective human leukocyte antigen alleles from long-term nonprogressors are associated with low HIV reservoir in central memory CD4 T cells. Clin Infect Dis **2012**; 54: 1495–503.
22. Buzon M, Sun H, Li C, et al. T Memory stem cells are a long-term reservoir for HIV-1 [Abstract #44]. In: 20th Conference on Retroviruses and Opportunistic Infections. Atlanta, GA, 3–6 March 2013.
23. Guadalupe M, Reay E, Sankaran S, et al. Severe $CD4^+$ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol **2003**; 77:11708–17.
24. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of $CD4^+$ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med **2004**; 200: 761–70.
25. Yukl SA, Gianella S, Sinclair E, et al. Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. J Infect Dis **2010**; 202:1553–61.
26. Yukl SA, Shergill A, Ho T, et al. The distribution of HIV DNA and RNA in cell subsets differs in gut and blood of HIV+ patients on ART: implications of viral persistence. J Infect Dis **2013**; 208:1212–20.
27. Descours B, Lambert-Niclot S, Mory B, et al. Direct quantification of cell-associated HIVDNA in isolated rectal and blood memory CD4 T cells revealed their similar and low infection levels in long-term treated HIV-infected patients. JAIDS,

J Acquired Immune Defic Syndr **2013**; 62: 255–9.
28. Walker BD, Hirsch MS. Antiretroviral therapy in early HIV infection. N Engl J Med **2013**; 368:279–81.
29. Chamberland A, Sylla M, Boulassel MR, et al. Effect of antiretroviral therapy on HIV-1 genetic evolution during acute infection. Int J STD AIDS **2011**; 22:146–50.
30. Evering TH, Mehandru S, Racz P, et al. Absence of HIV-1 evolution in the gut-associated lymphoid tissue from patients on combination antiviral therapy initiated during primary infection. PLOS Pathogens **2012**; 8:e1002506. doi: 10.1371/journal.ppat.1002506.
31. Gall A, Kaye S, Hue S, et al. Restriction of V3 region sequence divergence in the HIV-1 envelope gene during antiretroviral treatment in a cohort of recent seroconverters. Retrovirology **2013**; 10:8.
32. Hutter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med **2009**; 360:692–8.
33. Sallusto F, Lanzavecchia A. Memory in disguise. Nat Med **2011**; 17:1182–3.