REVIEW

Changes in HIV reservoirs during long-term antiretroviral therapy

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Purpose of review

To review current knowledge about the impact of long-term combination antiretroviral therapy (cART) on HIV reservoirs.

Recent findings

The number of HIV-infected cells that persist during long-term antiretroviral therapy is associated with the stage of HIV infection at the time of treatment initiation. Initiation of cART reduces the number of infected cells over the first 4 years of therapy, but thereafter there is no further decline despite long-term effective cART. The remarkable stability of infected cell numbers is likely due to a balance among homeostatic or antigen-driven proliferation of infected memory T-cells subsets, clonal expansion of a subset of infected cells as a consequence of specific retroviral integration sites, and death of other infected cells. At present, there is no effective means of accelerating the decay of infected cells in individuals initiated on cART during chronic HIV infection.

Summary

Given the stability and difficulty in eliminating HIV-infected cells, early initiation of cART in treatment-naïve HIV-infected patients is currently the most effective way to limit the size and diversity of HIV reservoirs.

Keywords

antiretroviral therapy, gut-associated lymphoid tissue, latent reservoir, residual viremia

INTRODUCTION

The most commonly encountered clinical scenario in the care of HIV-infected patients is the management of chronic HIV infection of unknown duration. Although enormous advances in HIV/AIDS treatment with highly potent, combination antiretroviral therapy (cART) have radically changed the natural history of the disease in the past 2 decades, eradication of HIV infection has remained elusive. Despite cART, low-level plasma viremia remains detectable by ultrasensitive HIV RNA assays in most patients [1,2], and a reservoir of latently infected CD4⁺ T cells persist, capable of being activated to produce infectious viruses [3–5], such that rapid rebound in plasma HIV RNA viremia occurs soon after treatment interruption [6,7]. The widely publicized report of the ‘Boston patients’, whose HIV infection relapsed despite prolonged cART-free viral remission with undetectable plasma HIV RNA and cell-associated HIV DNA after allogeneic stem cell transplantation, has further highlighted the difficulty in eliminating HIV reservoirs once they are established [8^{*}]. Here, we review the current knowledge about the impact of long-term

cART on HIV reservoir in patients who commenced treatment during chronic HIV infection, highlighting recent observations, and identifying key knowledge gaps about mechanisms of HIV persistence.

PERSISTENCE OF PLASMA HIV RNA ON COMBINATION ANTIRETROVIRAL THERAPY

The initiation of cART markedly reduces plasma HIV viruses from the blood stream to undetectable levels by commercially available assays in several phases of decay [9,10]. However, HIV RNA remains persistent in the peripheral blood at extremely low levels for years despite effective cART [1,2], with no further decline reported using the ultrasensitive single-copy assay after 3–5 years of treatment [11]. This suggests

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The case for early antiretroviral therapy (ART) towards a cure of HIV infection

KEY POINTS

- Despite the attrition of HIV-infected cells in the first few years of cART, the size of latent HIV reservoir on chronic cART is determined by the stage of HIV infection at the time of treatment initiation and is unaffected by intensification of cART with current antiretroviral agents.
- The size of this reservoir of latently infected CD4⁺ T cells may be up to 60-fold larger than previously assessed by VOA. Unfortunately, there is no efficient and accurate way to measure intact, replication competent HIV proviral population.
- The stability of this reservoir is maintained because of the infection of immature memory CD4⁺ T cells, such as T_{CM} and T_{SCM}, homeostatic proliferation and antigen-driven expansion of such CD4⁺ T cells, and clonal expansion of some infected cells as the result of specific retroviral integrations in host genes that influence cell proliferation and survival.
- Gut-associated lymphoid tissue plays a major role in long-term HIV persistence as a tissue reservoir of HIV-infected cells despite early cART.
- At present, early initiation of cART remains the best way of limiting the size of latent HIV reservoir.

the presence of a transcriptionally active reservoir of HIV-infected cells that continues to produce viruses. This reservoir appears to be remarkably stable, as several studies [12–14] that have added antiretroviral agents to the standard cART did not reduce the levels of residual viremia.

The set point of residual viremia on cART during this apparent plateau may be determined early by the state of HIV infection at the time of treatment initiation. A recent analysis of plasma HIV RNA levels by single-copy assay in 334 patients at weeks 192 and 208 of suppressive cART demonstrated that detectable plasma viremia is most strongly correlated with higher pre-ART HIV RNA [15[■]]. The analysis also found that higher on-treatment CD8 cell count and lower on-treatment CD4/CD8 ratio significantly correlate with detectable plasma HIV RNA, even after controlling for pre-ART HIV viremia, suggesting ongoing interaction between residual virus production and prolonged immune activation.

REDUCTION IN HIV-INFECTED CELL NUMBERS

As with the persistence of HIV RNA in the plasma, HIV DNA remains detectable in the peripheral blood mononuclear cells (PBMCs) in patients on cART

despite initial decay by as much as 10-fold [16–18]. The scale of this decay pales in comparison with that of plasma HIV RNA, which generally decreases by 4–5 log₁₀ during the first year of ART, suggesting that vast majority of HIV-infected cells persisting on cART contributes little, if anything, to plasma viremia. The majority of HIV DNA is thought to reside in resting CD4⁺ T cells during chronic viral suppression on cART, as confirmed by one study [19[■]] of treatment-naïve patients starting on raltegravir-based cART. The study showed that integrated HIV DNA within HLA-DR⁺ CD38⁺ resting memory CD4⁺ T cells decays in a slow and monophasic fashion during the first year of treatment, with half-life of 433 days. Interestingly, HIV DNA persisted in the activated CD38⁺ memory CD4⁺ T cells at levels comparable to that in resting cells, although the expression of proliferation marker Ki-67 was much higher, suggesting ongoing replenishment of HIV-infected cells in the activated CD4⁺ T cell compartment despite high cell turnover rate. Whether this replenishment occurs as a result of ongoing viral replication or proliferation of activated cells requires further elucidation.

HIV DNA can be used to assess the number of cells carrying integrated proviruses during long-term cART. Several studies [17,20,21] have shown that intermediate and aborted forms of HIV genetic integration, such as linear cDNA and 2-LTR circles, respectively, are preferentially lost during the initial phases of decay in patients on cART. Serial detection of PBMC-associated HIV DNA in three well suppressed patients on effective cART found that the ratio of total to integrated HIV DNA approached one within 1 year of starting treatment, suggesting that HIV DNA mainly exists as integrated proviruses in patients who are well controlled on treatment [22]. Given that the majority of HIV-infected cells contains only one copy of HIV DNA [23,24[■]], the measurement of total HIV DNA in chronically suppressed patients provides a simple and reasonably reproducible estimate in the number of HIV-infected cells. As described below, however, because most infected cells contain defective proviruses and the proportion of intact proviruses varies across individuals, HIV DNA is not a good measure of the replication-competent proviral reservoir.

The long-term durability of the HIV proviruses is illustrated by a study [25[■]] that tested PBMCs from 30 HIV-infected patients on effective cART for up to 12 years. Beyond 4 years of treatment, no more appreciable decline was observed in PBMC-associated HIV DNA, regardless of their age, treatment regimens, and/or systemic immune activation. Interestingly, the level of total HIV DNA, even after 10 years of treatment, is still strongly correlated

with total HIV DNA at baseline, again suggesting a correlation between the stage of HIV infection at the time of treatment initiation and the number of infected cells after long-term cART. The association of HIV DNA levels on treatment with the stage of HIV infection before treatment initiation has also been suggested by a number of cross-sectional studies [26[■],27] of virally suppressed patients on cART.

IMPACT ON LATENTLY INFECTED RESTING CD4⁺ T CELLS

Although HIV DNA levels in PBMCs provide a reasonable estimate of the number of infected CD4⁺ T cells, the vast majority of these proviruses is defective and incapable of viral replication [28], making cell-associated HIV DNA an unsuitable measure of the latent HIV reservoir. More direct measure of HIV latency is provided by the viral outgrowth assay (VOA). Although labor-intensive and time-consuming, VOA has been a gold standard in estimating infectious virus production in latently infected CD4⁺ T cells. The long-term stability of these latently infected resting CD4⁺ T cells is highlighted in a longitudinal study of 62 HIV-infected patients who were suppressed on effective cART for as long as 7 years. The frequency of latently infected cells remained stable between 0.03 and 3 infectious units per million resting CD4⁺ T cells, with decay half-life of 44 months, indicating that cART alone cannot eradicate HIV in patients who initiate therapy during chronic HIV infection [29]. Using this technique, it was estimated that one in 10⁶ resting CD4⁺ T cells is latently infected with replication-competent HIV [30].

However, a more recent study by Ho *et al.* [31[■]] suggested that the size of latent HIV reservoir may be far greater than that provided by VOA. In this study, 213 noninduced proviral clones from VOA assay were analyzed. Although the majority (88.3%) of HIV proviruses contained identifiable defects that preclude viral replication, the remainders were intact genomes, and proviral clones reconstructed from these sequences were fully replication competent. Moreover, most of the intact proviruses had unmethylated promoters and were integrated into active transcription units, retaining the potential for activation. Indeed, upon a second round of T cell activation, nearly 25% of the wells that were negative for virus replication became positive, suggesting that even with maximal stimulation, the induction of latent proviruses is still unpredictable and likely stochastic. These studies illustrate the formidable challenges facing any effort to eradicate all intact proviruses through activation of proviral expression.

TISSUE RESERVOIRS IN PATIENTS ON COMBINATION ANTIRETROVIRAL THERAPY

The source of residual viremia on cART remains undefined but is likely from multiple tissue sources [32,33]. In-situ hybridization of patient lymphoid tissues confirmed the presence of active HIV expression despite suppression of plasma viremia below the limit of clinical detection [34]. Similarly, HIV DNA and RNA are readily detected in gut-associated lymphoid tissue in chronically infected patients despite effective cART [35–37]. This is not surprising, as gut-associated lymphoid tissue constitutes the largest reservoir of CD4⁺ T cells in the body and is profoundly affected early in the course of acute HIV infection [38]. However, the extent to which HIV-infected CD4⁺ T cells in the gut compartment are in equilibrium with the cells in peripheral blood is unclear. Although measurements of cell-associated HIV DNA and unspliced mRNA levels in total CD4⁺ T cell from duodenum, ileum, ascending colon, and rectum were reported to be significantly higher than those in the peripheral blood [37], the difference did not reach statistical significance in a separate study [39[■]] comparing levels of HIV DNA in rectal and peripheral blood memory CD4⁺ T cells. Some of this inconsistency may be explained by different distribution of CD4⁺ T-cell subsets between peripheral blood and various sites of the gut – although CCR7⁺ ‘lymphoid-homing’ central memory (T_{CM}) and naïve CD4⁺ T cells accounts for more than 50% of CD4⁺ T cells in the blood, thereby comprising a larger proportion of total HIV DNA, more effector memory (T_{EM}) and to a lesser extent, transitional memory (T_{TM}) CD4⁺ T cells, are found in the gut. In addition, there appears to be some interpatient variability in HIV DNA content among various subsets of memory CD4⁺ T cells [40[■]].

The presence of HIV reservoirs in other cellular lineages and tissue compartments is less well studied. Several studies [40[■],41] have reported the presence of HIV DNA in myeloid cells from the gut of virally suppressed patients on cART. Alveolar macrophages have also been reported to harbor HIV DNA [42[■]]. However, these reports have not been able to distinguish between phagocytosed HIV proviral DNA from other infected cells by macrophages and HIV infection of macrophages [43[■]]. By contrast, HIV infection of perivascular macrophages and microglial cells in the central nervous system is well documented in viremic patients, although whether the brain constitutes a discrete HIV reservoir in virally suppressed patients on long-term cART is uncertain [44].

The case for early antiretroviral therapy (ART) towards a cure of HIV infection

POTENTIAL MECHANISMS OF HIV PERSISTENCE

It is unclear whether the levels of HIV-infected cells in patients on suppressive cART are maintained because of intrinsic capacity of these cells for prolonged survival and/or proliferation, or by continued replenishment due to low-level HIV viral replication. Potential mechanisms of HIV persistence are as follows:

- (1) replenishment by low-level HIV replication in viral sanctuary sites,
- (2) prolonged survival of HIV-infected cells without cell proliferation,
- (3) clonal expansion from homeostatic proliferation of infected CD4⁺ T cells,
- (4) clonal expansion from antigen-driven proliferation of infected CD4⁺ T cells,
- (5) clonal expansion from HIV integrations in host genes that affect cell proliferation and survival.

Some treatment intensification studies [45,46[■]] using the integrase inhibitor raltegravir showed transient increase in 2-LTR circles and reduced levels of unspliced HIV mRNA in the ileal biopsy specimens [47], suggesting that residual HIV viral replication may indeed be present. However, studies [32,48,49[■]] of HIV proviral genetics have not revealed evidence of viral genetic evolution on cART, and many other treatment intensification studies [12–14] have not shown an effect on the level of persistent viremia. Interestingly, according to a mathematical model of T cell dynamics in HIV infection proposed by Sedaghat *et al.* [50], HIV viral dynamics observed in patients on cART cannot exclude the possibility of low-level viral replication, but this is not expected to affect significantly the decay rate of the latent reservoir. Taken together, these findings suggest that stability of HIV-infected cells, rather than continued replenishment by low-level viral replication, is the major mechanism by which HIV infection persists.

Indeed, analysis of HIV DNA in various T-cell subsets by Chomont *et al.* [51] in 31 virally suppressed patients on cART found that long-lived memory CD4⁺ T cells, such as T_{CM} and T_{TM} cells, accounted for a higher fraction of the HIV proviruses in comparison to T_{EM}, terminally differentiated and naïve CD4⁺ T cells (mean 51.7, 34.3, 13.9, 1.9, and 0.3%, respectively). Specifically, more proviral DNA was detected in T_{CM} cells among patients with higher CD4 counts, whereas those with poorer immune reconstitution had more HIV DNA in T_{TM} cells, indicating variability across patients in T-cell subset infection [51]. Recently, a population of even more immature memory CD4⁺ T cells with

stem cell-like properties (T_{SCM}) has been described to harbor HIV DNA as well [52[■]]. Although T_{SCM} cells represent a minor fraction of total memory CD4⁺ T cells, contributing on average only 8% of the total HIV proviral reservoir in the study cohort, their stem cell-like properties confer even greater capacity to proliferate and survive. When historical samples from the first year of cART were compared with those taken later in the treatment course (range: 7–11 years) in a subset of eight patients, T_{SCM} cells showed less steep decline in cell-associated HIV DNA compared with the more mature T_{EM} and terminally differentiated subsets. The capacity of T_{CM} and T_{SCM} cells for prolonged survival and self-renewal through antigen-driven or homeostatic proliferation is likely an important factor in the long-term stability of HIV infection. Overcoming this intrinsic cellular longevity is a critical required component of cure strategies.

Recent studies [53[■],54[■]] have also suggested that significant survival and proliferative advantage could be conferred through specific integration sites by HIV DNA. For example, Maldarelli *et al.* [54[■]] analyzed 2410 integration sites in a small cohort of five HIV-infected patients and found that integrations in certain genes, such as *MKL2* and *BACH2*, were clearly overrepresented and associated with cellular persistence and clonal expansion. Although these studies are limited by the small number of patients included, selective expansion of cellular clones as a result of specific integration sites offers an intriguing explanation for the perceived changes in population structure of HIV proviral reservoir from previous phylogenetic studies [32,49[■]]. In addition, further characterization of these cellular clones may offer unique targets to reduce the number of HIV-infected cells. The impact of cART on the genetics of HIV persistence is reviewed in more detail by Kearney *et al.* in this issue.

CONCLUSION

Longitudinal studies in patients who started suppressive cART during chronic infection have highlighted the extraordinary capacity of HIV infection to persist. Although attempts are ongoing to reduce HIV-infected cell numbers and the size of the latent HIV reservoir, no approaches have been shown to effectively reduce the size of this reservoir [55[■]]. By contrast, there is strong evidence that the number of HIV-infected cells that persist on cART is determined, at least partially, by the timing of treatment initiation [15[■],25[■]], with earlier initiation reducing infected cell number [38,56–58]. There is also accumulating evidence that very early cART may restrict seeding of T-cell subsets [59[■],60[■]], decrease

viral diversity [61[■],62], and increase the likelihood of posttreatment immunologic control [60[■]] (reviewed in more detail by Ananworanich *et al.*, Rouzioux *et al.*, and Lewin *et al.* in this issue). Therefore, treatment initiation at the earliest possible stage of infection is currently the only way to limit the size of HIV-infected cells.

Additional research is needed to define the mechanism of HIV persistence. Although prolonged survival of HIV-infected cells without proliferation remains a possibility, HIV-infected cells can also undergo clonal expansion through either antigen-driven or homeostatic proliferation, or integration of HIV proviruses into sites that increase cell proliferation or survival. Better understanding of these mechanisms will improve the specificity with which infected cells can be targeted. More studies are also needed to identify HIV tissue reservoir(s) and their role in maintaining residual viremia and serving as a source of viral rebound after treatment cessation. Finally, cheaper, more efficient and more accurate quantitative methods are needed to assess and follow the size of the latent HIV reservoir to precisely measure the efficacy of treatment strategies aimed at reducing the number of latently infected cells.

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Conflicts of interest

J.W.M. is a consultant to Gilead Sciences and holds share options in RFS Pharmaceuticals. No other conflicts are reported. Other author reports no potential conflicts.

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