HIV reservoirs: pathogenesis and obstacles to viral eradication and cure

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Plasma HIV viremia can be suppressed and maintained below the limits of detection for prolonged periods of time in the vast majority of HIV-infected individuals who receive antiretroviral therapy (ART). Thus, the clinical outcome for HIV-infected individuals who have access to these drugs is dramatically improved. However, ART alone cannot eradicate HIV in infected individuals and this impediment is likely in part due to the persistence of viral reservoirs in the peripheral blood and lymphoid tissues of infected individuals despite the suppression of plasma viremia. In recent years, major research efforts have been dedicated to a better understanding of the pathogenesis of persistent HIV infection and to the development of therapeutic strategies aimed at eradicating virus in infected individuals receiving ART. In this review, we discuss the pathophysiology of CD4+ T-cell HIV reservoirs, including recent advances in our understanding of the mechanisms of persistent viral infection and perspectives for eradication of HIV in infected individuals.

Keywords: antiretroviral therapy, cure, eradication, HIV, viral reservoirs

Introduction

Antiretroviral therapy (ART) dramatically suppresses HIV replication in the vast majority of infected individuals, resulting in a substantial decline in morbidity and mortality in both developed and developing nations where drugs are available [1–7]. Shortly after the wide availability of combination ART involving at least three drugs in 1996 and the demonstration that plasma viremia could be rapidly and profoundly suppressed to below the level of detection, optimism grew in the field that HIV could potentially be eradicated in infected individuals receiving ART for a few years [8]. However, the prospect for eradication of HIV diminished considerably in 1997 following the publication of three independent studies in which the persistence of a small but detectable pool of latently infected, resting CD4+ T cells carrying replication-competent virus was documented in virtually all study patients who had received clinically effective ART for up to 3 years [9–11]. Moreover, a number of studies demonstrated rapid viral rebound shortly after discontinuation of therapy in infected individuals in whom profound and sustained suppression of plasma viremia had been achieved for prolonged periods [12–17]. This finding casts doubt on the feasibility of eradication of HIV by ART alone. These sobering observations subsequently led to the intensified pursuit of new avenues of research with a focus on the cellular and molecular characterization of the latent HIV reservoir and the development of strategies designed to eliminate persistently infected CD4+ T cells in individuals receiving ART.

Following the initial observation over a decade ago that latently infected, resting CD4+ T cells persist in infected individuals receiving clinically effective ART [9–11, 18,19], considerable progress has been made regarding our understanding of the pathogenesis of HIV reservoirs in the era of successful ART (Fig. 1). However, the...
prospects for eradicating HIV in a sizable proportion of infected individuals receiving ART remain unclear and will likely remain so until the precise mechanisms by which persistent HIV reservoirs are established and maintained are fully understood. In addition, the sources of persistent infection and rebounding plasma viremia upon discontinuation of therapy need to be identified as well as the mode of viral transmission/spread of infection within lymphoid tissues. Finally, the development of clinically relevant, novel therapeutic agents targeting persistently infected cells must be assessed in infected individuals receiving ART.

This article will review the state of our knowledge of CD4+ T-cell-associated HIV reservoirs and discuss recent therapeutic developments aimed at eradicating the virus in infected individuals.

The latent HIV reservoir in resting CD4+ T cells: a major impediment to the eradication of virus

The presence of latently infected, resting CD4+ T cells carrying integrated HIV DNA in infected individuals was first demonstrated in 1995 [20], 2 years prior to the report of effective ART in the clinical setting [21,22]. However, the concept of HIV latency had been previously introduced on the basis of the studies involving the establishment of HIV-infected cell lines expressing low levels of virus [23] and the use of various cytokines [24] and transcription factors [25] to reverse this state of viral latency. Subsequently, primary resting CD4+ T cells carrying unintegrated HIV DNA (preintegration latency) were shown to generate infectious virus upon cellular activation in vitro [26] and ex vivo [27]. Then, the concept of postintegration latency was introduced in a report in which a very small fraction of resting CD4+ T cells in viremic HIV-infected individuals were shown to carry the integrated form of viral DNA in the genome of the host cells [20]. This study was followed by a comprehensive analysis of the frequency of resting CD4+ T cells carrying integrated HIV DNA that were capable of producing replication-competent virus upon cellular activation in both the blood and lymphoid tissues of untreated infected individuals [28]. This study estimated that the total body burden of the latent viral reservoir was very small (fewer than 10 million cells per infected person) and suggested at least two reasons why such infected cells could persist for extended periods in vivo. First, the latently infected, resting CD4+ T cells exhibited primarily a memory phenotype (CD45RO+), implying that such infected cells could be long-lived. Second, resting CD4+ T cells carrying infectious HIV could do so without expressing viral antigens on their cell surface, thereby enabling them to escape recognition by the host immune system and to resist virus-induced cytopathic effects. Indeed, several follow-up studies demonstrated that the latent viral reservoir carrying replication-competent HIV persists in virtually all infected individuals receiving clinically effective ART. This observation casts serious doubt on the feasibility of eradicating virus by therapy alone [9–11,18,19].

Having established that the latent viral reservoir persists in infected individuals receiving ART despite profound and sustained suppression of plasma viremia, a series of studies was launched in the late 1990s and early 2000s with a goal of better understanding the pathophysiologic mechanisms involved in the establishment and maintenance of HIV latency. In particular, in anticipation of the possibility that early initiation of ART may prevent the generation of viral latency, studies were undertaken to determine the approximate length of time required for establishing a pool of latently infected, resting CD4+ T cells. It became evident that the latent HIV reservoir establishes itself very early during the course of infection. This conclusion was based on the observation that initiation of ART as early as 10 days following the onset of clinical symptoms associated with primary HIV infection.
did not prevent the establishment of a pool of latently infected, resting CD4$^+$ T cells carrying replication-competent virus [29]. In addition, the latent viral reservoir was shown to expand rapidly following the re-emergence of plasma viremia after discontinuation of ART in HIV-infected individuals with undetectable plasma viremia [30]. There are a number of challenges associated with both identifying individuals during primary HIV infection [31] and initiating ART prior to the appearance of high levels of plasma viremia [29]. Nonetheless, several studies have attempted to determine the half-life of latently infected, resting CD4$^+$ T cells and the duration of ART required to eliminate these infected cells in early-treated individuals. Two longitudinal studies demonstrated that the half-life of the latent viral reservoir was approximately 4–6 months in HIV-infected individuals who initiated ART during the acute/early phase of infection [32,33]. These studies projected that it would require at least 7–8 years of continuous therapy to eradicate HIV using the assumptions that ART was 100% effective at suppressing viral replication and that no other viral reservoirs existed in vivo [32,33]. These predictions were in sharp contrast to other longitudinal studies that primarily focused on infected individuals who initiated ART during the chronic phase of infection and in which the half-life of the latent viral reservoir exceeded 44 months. This observation suggested that even 60 years of ART may not be sufficient to completely eradicate HIV [18,19].

Assuming that the latent viral reservoir persists in virtually all HIV-infected individuals receiving ART and that this reservoir is considered to be one of the major impediments to eradicating HIV [9–11,18,19], major efforts have been directed towards elucidating the mechanism(s) by which these infected cells persist in vivo. A number of cellular and molecular mechanisms that could explain HIV persistence have been proposed over the years. These include a paucity of cellular factors required for robust HIV expression in infected cells [34,35], modification of chromatin structure that prevents viral replication [36,37], methylation of the S' long terminal repeat region of HIV proviral DNA [38,39], interference of HIV expression by the proximal host gene promoters [40,41], homeostatic proliferation regenerating latently infected cells in the absence of cell death [42], and reversion of productively infected, activated CD4$^+$ T cells to the resting memory state without any specific mechanisms [28]. Most likely, more than one of these, or other yet to be identified mechanisms, may contribute to the maintenance of the latent viral reservoir in vivo.

Persistence of HIV in infected individuals receiving antiretroviral therapy: beyond the latent viral reservoir

Although the pool of latently infected, resting CD4$^+$ T cells has been the most extensively studied HIV reservoir to date and is widely recognized as one of the major obstacles to achieving eradication of virus [43,44], low levels of HIV replication may persist in infected individuals despite their treatment with clinically effective ART [32,45–55]. In recent years, the lack of consensus on the life span of the latent viral reservoir [19,32,33] has sparked an intense debate regarding the possibility that low levels of HIV replication in subsets of CD4$^+$ T cells in the lymphoid tissue may contribute to replenishment of a pool of infected resting CD4$^+$ T cells. By this mechanism, the overall half-life of the viral reservoir could be indefinitely extended. In this regard, previous mathematical models have suggested that productively infected CD4$^+$ T cells have a relatively short in-vivo half-life (<1 day) following initiation of ART [8,56]. The data indicate that such cells should no longer be present in infected individuals who had been receiving clinically effective ART for extended periods. However, activated CD4$^+$ T cells, enriched at high purity from the blood of a viremic individual receiving ART, carry HIV proviral DNA and are capable of spontaneously producing virions in culture [57]. Furthermore, in this study, phylogenetic analyses of HIV env provided evidence for virologic cross-talk between infected resting and activated CD4$^+$ T cells in the absence of detectable plasma viremia [57]. These findings raise the possibility that ongoing HIV replication may persist in nonresting CD4$^+$ T-cell reservoirs in aviremic individuals.

Without treatment, HIV replicates primarily in the lymphoid tissues [58,59] with extremely high levels of viral replication [60–62] and extensive destruction of CD4$^+$ T cells [63–65] in the gut-associated lymphoid tissue (GALT) of infected animals and humans. Recent studies have therefore shifted focus to the GALT as a major viral reservoir in infected individuals receiving ART. Such studies have demonstrated that the overall HIV burden is substantially higher in the GALT than in the blood of infected individuals receiving ART for long time periods [66–69].

Collectively, these findings suggest that low levels of ongoing viral replication, along with the persistence of HIV in the latent viral reservoir in the blood, may occur in the absence of detectable plasma viremia (<50 copies of HIV RNA). Therefore, strategies targeting productively infected cells in the tissue compartment must be developed to further decrease the number of infected cells. The recent introduction of newer classes of antiretroviral drugs [70,71] (72], this issue) combined with the development of highly sensitive assays that are capable of detecting plasma-associated HIV RNA at the single copy level (below 50 copies of HIV RNA per ml of plasma) [73–76] should play an important role in studying and addressing the issue of persistent virus reservoirs. In this regard, intensification of conventional ART with newer classes of drugs was considered a possible approach to further reduce the residual pool of virus if low levels of
ongoing viral replication were indeed occurring in treated infected individuals. Unfortunately, this line of investigation has led to further disagreement. On the one hand, several recent studies have demonstrated that intensification of ART does not further reduce residual plasma viremia [77–80]. Therefore, ongoing HIV replication did not appear to occur and reactivation of the latent viral reservoir seemed to be the main mechanism by which residual plasma viremia is maintained in infected individuals receiving ART. On the other hand, two studies taking a different approach have demonstrated an accumulation of circualized HIV DNA and a significant reduction in the level of cell-associated RNA in the blood [81] and GALT [82], respectively, upon intensification of ART in aviremic individuals. Furthermore, evidence for cell-to-cell transmission of HIV in the presence of antiretroviral drugs has been recently demonstrated [83]. This finding implies that low levels of ongoing HIV replication in the tissue compartment may not be detectable as plasma viremia. Given the conflicting data regarding the virologic outcome of intensification of ART, clinical studies involving comprehensive immunologic and virologic analyses of a large number of study participants will be necessary to better delineate the role of residual HIV replication in infected individuals receiving ART. In addition, the precise origin and clinical importance of residual plasma viremia need to be further elucidated to better understand the dynamics of HIV infection in the setting of effective ART.

**Therapeutic strategies for the eradication of persistent viral reservoirs in HIV-infected individuals receiving antiretroviral therapy: bench to bedside**

A major thrust of HIV therapeutic research over the past few years has been the development of clinical strategies aimed at eradicating the virus in infected individuals who receive ART. The pursuit of a cure for HIV disease began in earnest over a decade ago with strategies that directly or indirectly stimulated latently infected, resting CD4+ T cells in vivo. These studies were based on the assumption that activation of the latent viral reservoir would result in rapid cell death due to HIV-induced cytopathic effects and that the infectious virus released by these activated cells would be contained by the administration of ART. In this regard, it has been shown that a combination of cytokines, namely IL-2, IL-6, and TNF-α, can induce HIV replication from the latent viral reservoir ex vivo [35]. Indeed, several clinical studies have attempted to purge the latent HIV reservoir in infected individuals receiving ART using immune-activating agents, such as IL-2 [84] and anti-CD3 antibody [85,86]. Among these approaches, one nonrandomized study demonstrated a marked diminution of the size of the pool of latently infected, resting CD4+ T cells in infected individuals receiving ART with repeated cycles of intermittent IL-2 compared with those who received ART alone [84]. Of note, no resting CD4+ T cells carrying replication-competent HIV could be detected in the blood and lymphoid tissue of these patients [84]. Nonetheless, rapid rebound of plasma viremia was observed following empiric interruption of ART in these patients [30]; similar rebounds of viremia following interruption of ART were seen in patients treated with anti-CD3 antibody with ART [86]. Taken together, these results strongly suggest that strategies involving activation of the latent HIV reservoir with potent T-cell stimulators may not be sufficient to purge the virus from infected, resting CD4+ T cells and thus achieve total eradication of HIV infection.

In recent years, molecular studies on the persistence of HIV in latently infected cells have suggested that repression of chromatin structure may play a role in the inhibition of HIV transcription and that inhibitors of histone deacetylases (HDACs) may allow expression of viral RNA from the latent viral reservoir [87,88] (Table 1). One of these HDAC inhibitors, valproic acid (VPA), has been tested extensively in vitro and ex vivo as a potential candidate for purging the latent HIV reservoir. One study demonstrated a diminution of the size of the latent viral reservoir in a small number of infected individuals receiving ART [98]. However, several subsequent studies have demonstrated no measurable effect of VPA on the frequency of resting CD4+ T cells carrying latent HIV in vivo [89–91]. These conflicting results may be due in part to the fact that HDAC inhibitors are not potent stimulators of HIV expression in latently infected, resting CD4+ T cells and do not promote T-cell activation. Currently, different HDAC inhibitors are being tested in clinical trials in order to investigate whether more potent agents, such as suberoylanilide hydroxamic acid [99], are better capable of purging the latent virus.

In addition to the aforementioned therapeutic agents that target the latent HIV reservoir, the modification of host genetics for the purpose of generating HIV-resistant CD4+ T cells is under active investigation (Table 1). Such an approach was inspired by a recent case report from Germany describing an HIV-infected patient with acute myeloid leukemia who underwent multiple rounds of chemotherapy and stem cell transplants from a CCR5Δ32 homozygous donor. The virus appears to be completely eliminated in this individual [96,97]. Remarkably, the plasma viremia of this patient did not rebound following discontinuation of ART and his viral load has remained fully suppressed for several years in the absence of ART [96,97]. On the basis of the initial success in a mouse model [93,100], a number of clinical trials have been recently launched in which autologous CD4+ T cells are treated with a zinc-finger nuclease that is designed to specifically eliminate or reduce the expression of HIV coreceptors CCR5 or CXCR4 ex vivo [94,95]. These genetically modified, HIV-resistant CD4+ T cells were
then transfused back into the autologous donors. Preliminary findings have demonstrated a repopulation and steady increase in modified CD4+ T cells in the blood and lymphoid tissues in some study participants [94,95]. However, the fate of preexisting HIV reservoirs in such patients remains to be determined as does the question of whether chemotherapy will be necessary to eliminate the wild-type CCR5+/CXCR4+ CD4+ T cells that may harbor infectious virus prior to transfusion of genetically modified cells. Future clinical trials involving long-term follow-up and discontinuation of ART will shed light on the feasibility of HIV eradication using these novel, more targeted genetic approaches.

**Conclusion**

Despite more than a decade of intense research and recent therapeutic advances, achieving a true eradication of HIV remains a daunting challenge for the HIV/AIDS scientific community. The following key issues must be addressed and implemented by the scientists in the field if the eradication of HIV in infected individuals is to someday become a realistic goal (Fig. 2). First, it is necessary to gain a better understanding of the immunologic and virologic factors/mechanisms that contribute to the persistence of HIV in infected individuals receiving ART. In particular, the following need to be fully delineated: the precise mechanism by which the latent viral reservoir is established and maintained; the role of tissue-derived productively infected cells in the persistence of virus; the origin of rebounding plasma viremia upon discontinuation of therapy; and the effect of immune activation on viral burden in infected individuals receiving ART.

Second, the impact of the use of aggressive ART regimens administered as early as possible in the course of HIV infection on the establishment and size of the HIV reservoir and on our ability to ultimately eradicate this reservoir needs to be addressed. Third, clinically relevant and reproducible high-throughput virologic assays need to be developed in order to measure the efficacy of agents that are designed to target persistent HIV reservoirs. Fourth, strategies aimed at targeting both latent (purging agents) and nonlatent viral reservoirs (HIV-specific killing agents such as immunotoxins) may need to be pursued simultaneously in order to eradicate HIV. Given that single cells carrying infectious HIV are potentially capable of re-igniting viral replication in the absence of antiretroviral drugs, a variety of cell types including cells of monocyte/macrophage lineage in tissue compartments, such as the lymph nodes and other organs, need to be examined and fully characterized as a potential sanctuary for the virus. Finally, eradication strategies may need to include immune-based therapies, such as therapeutic vaccination [92] that are capable of boosting the host immune responses against HIV and thus contribute to the eradication process.

**Table 1. Current therapeutic strategies aimed at eradicating HIV in infected individuals receiving antiretroviral therapy.**

<table>
<thead>
<tr>
<th>Approach</th>
<th>Agent/drug</th>
<th>Target cells</th>
<th>Mode of action</th>
<th>Challenges</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Elimination of latent HIV reservoir</td>
<td>HDACi (VPA, SAHA)</td>
<td>Latently infected resting CD4+ T cells</td>
<td>Induction of HIV expression</td>
<td>Minimal impact on productively and recently infected CD4+ T cells</td>
<td>[89–91]</td>
</tr>
<tr>
<td>Enhancement of anti-HIV immunity</td>
<td>Therapeutic vaccine</td>
<td>Latently and/or productively infected CD4+ T cells</td>
<td>Cytolytic CD8+ T-cell-mediated killing upon recognition of viral antigen</td>
<td>Ineffectiveness on the latent viral reservoir</td>
<td>[92]</td>
</tr>
<tr>
<td>Gene therapy</td>
<td>Zinc finger nuclease-mediated elimination of HIV-encoding co-receptors</td>
<td>CD4+ T cells</td>
<td>Generation of HIV-resistant CD4+ T cells</td>
<td>Unknown fate of viral reservoir post-transfusion with genetically modified cells</td>
<td>[93–95]</td>
</tr>
<tr>
<td>Stem cell transplantation</td>
<td>Bone marrow transplantation from CCR5Δ32 homozygous donor</td>
<td>CD4+ T cells</td>
<td>Generation of HIV-resistant CD4+ T cells</td>
<td>Need for chemotherapy prior to transplantation</td>
<td>[96,97]</td>
</tr>
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HDACi, histone deacetylase inhibitor; SAHA, suberoylanilide hydroxamic acid; VPA, valproic acid.
Fig. 2. Potential strategies for eradicating HIV in infected individuals receiving antiretroviral therapy.

Considering that a true sterilizing cure will be very difficult to achieve in a large proportion of infected individuals even in the best of circumstances involving early access to therapy, the possibility of a ‘functional’ cure should be pursued. In this case, the virus would not be entirely eradicated, but HIV-specific immune responses are boosted to prevent the rebound of plasma viremia upon discontinuation of ART. The early initiation of ART following acute infection would enhance the possibility of such an immune–based functional cure by minimizing the size of the viral reservoir. The maintenance of this functional cure may likely require multiple rounds of immune enhancement over several years to ensure that the small amount of residual HIV in the absence of ART is not re-ignited in the absence of ART.

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Conflicts of interest
There are no conflicts of interest.

References


42. Lenas T, Contreras X, Peterlin BM. Transcriptional interference antagonizes proviral gene expression to promote HIV latency. Cell Host Microbe 2008; 4:123–133.


NF-κB p50 promotes HIV latency through HIV-1 replication and immune dynamics are T cells in HIV-1-infected patients receiving ART. J Infect Dis 2010; 202:153–156.


Vella S, Schwartlander B, Sow SP, Eholie SP, Murphy RL. The history of antiretroviral therapy and its implementation in resource-limited areas of the world. AIDS 2012; 26:1231–1241.


Heg M, Margolis DM. Counterregulation of chromatin deacetylation and histone deacetylase occupancy at the integrated promoter of human immunodeficiency virus type 1 (HIV-1) by the HIV-1 repressor Y11 and HIV-1 integrator Tat. Mol Cell Biol 2000; 20:2963–2973.

Williams SA, Chen LF, Kwon H, Ruiz-Jarabo CM, Verdin E, Greene WC. NF-kappaB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. EMBO J 2006; 25:139–149.


