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Challenges and Strategies for the Eradication of the HIV Reservoir

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Abstract

Despite the success of highly active antiretroviral therapy (HAART) for inhibiting HIV replication and improving clinical outcomes, it fails to cure infection due to the existence of a stable latent proviral reservoir in memory $CD4^+$ T cells. Because of the longevity of these cells harboring transcriptionally silent proviruses, devising strategies to induce viral gene expression so the host immune response can mediate clearance of the infected cells or the cells can undergo virus-induced cell death, has been of considerable recent interest. Here, we review current knowledge of latency, and the challenges to virus induction and eradication. Novel strategies to reactivate HIV reservoirs more effectively, in combination with immunotherapy, could lead to better clearance of the latent HIV reservoir.

Introduction

Resting $CD4^+$ memory T cells are the main reservoir harboring latent integrated HIV-1 during highly active antiretroviral therapy (HAART) [1,2]. Because of their long lifespan and quiescent status, latently infected resting $CD4^+$ T cells are particularly efficient at escaping immune surveillance and represent a major obstacle to curing HIV infection. Indeed, patients who have been on suppressive HAART for long periods rapidly demonstrate rebounds in viral load during treatment interruptions. Recent efforts have focused on reactivating the latent viral reservoirs in the setting of HAART with the hope that viral cytopathic effects or the cellular immune response will kill the infected cells [3,4]. However, current methods of activating latently integrated virus have not been proven to be effective at inducing virus expression to levels sufficient for inducing death of the infected cells, and the host immune response may be insufficiently activated to clear infection [5,6]. Furthermore, initial trials with agents to reverse latency have not demonstrated a reduction in the viral reservoir [7]. Thus, identifying a method to prime cells expressing reactivated virus to die more readily may be essential for eradicating the latent viral reservoir. Here, we provide an overview of HIV latency in CD4 T^+ cells during HAART, review challenges to clearing the

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latent reservoir, and discuss alternative approaches to the reactivation and eradication of latently infected cells.

HIV infection and latency

HIV latency refers to a highly stable and transcriptionally silent integrated proviral DNA reservoir within resting memory $CD4^+$ T cells that can produce infectious virus when the host cell is reactivated by antigen or during interruptions in HAART [8]. Latency is likely established early during acute HIV-1 infection of the host [9] and as a result of the cellular tropism of HIV, which optimally replicates in activated $CD4^+$ T cells.

Exposure to antigens leads to activation and expansion of antigen-specific effector T cells, most of which are removed by programmed cell death after the immune response subsides [10,11]. However, a small number of these antigen-specific T cells survive and develop into memory cells [12,13]. The selective retention of antigen-specific memory T cells and ability to revert to a resting state and persist after an immune response is crucial for the maintenance of long-term immunological memory [14,15]. However, this longevity and quiescence makes them a perfect host for perpetuating latently integrated proviruses [1]. With regulatory features highly adapted to the environment of T cells, HIV appears to capitalize on the process of memory T cell development for the establishment of latency. While most of the activated $CD4^+$ T cells that become infected are rapidly killed, it is thought that the rare, activated $CD4^+$ T cells that are infected as they transition to a resting memory state survive. In support of this idea, latent HIV has been found in resting memory $CD4^+$ T cells but not naïve $CD4^+$ T cells [16-19], and these cells account for only 1 in 10^6 of resting $CD4^+$ T cells [18,19]. However, reactivation of these infected $CD4^+$ T cells rekindles viral replication.

Persistence of the reservoir of latently infected cells

How the persistent HIV $CD4^+$ T cell reservoir in the host is maintained remains incompletely understood. A number of past studies indicate that long-term HAART eventually halts viral evolution in the host, suggesting that viral replication is largely suppressed. Under these circumstances, the occasional blips in measurable plasma viremia may result from antigen activation of infected T cells. HIV DNA integrant frequencies remain stable over time and a large portion of the virus appears to be clonal in nature [20,21]. Indeed, two recent studies demonstrate that proliferation of latently HIV-infected $CD4^+$ T cells may play a key role in maintaining this durable viral reservoir [22,23]. In both papers, the authors observed in well-suppressed patients clonal outgrowth of cells with HIV integrated in or near a small set of cellular genes. Interestingly, some of the genes are known to be involved in tumorigenesis or cell cycle control. In particular, BACH2 is a frequent site of integration [22-24]. BACH2 is involved in T cell development and cytokine production [25,26], suggesting that integration within this gene may influence regulation of proliferation. Importantly, these clonally expanded populations of $CD4^+$ T cells with integrated provirus can produce infectious HIV [27].

Other studies indicate the existence of sanctuary sites within the infected patient on HAART. As most earlier studies on genetic diversity of HIV during HAART have focused on blood derived variants, questions have remained about whether those results necessarily apply to other compartments within the body. Of particular interest are lymphoid tissues, where the frequency of infected cells is higher [28], and the intracellular concentration of antiretroviral drugs is relatively low [28a]. A recent study found that virus evolution may continue in lymphoid tissues of patients with undetectable levels of virus in blood [29], suggesting that improving antiretroviral drug penetration into lymphoid tissues may be necessary to halt viral replication and eliminate the viral reservoir. However, even if antiretroviral drug concentrations in lymph nodes can be increased, it is unclear how this would improve clearance of latently infected cells in this reservoir if homeostatic proliferation of latently infected cells contributes to maintaining the viral reservoir.

Latency Reactivation and Latency Reversing Agents

Although the mechanisms involved in latency in $CD4^+$ T lymphocytes are incompletely understood, it is believed that multiple mechanisms act in concert to establish and maintain latency. Transcriptional interference by cellular genes at the site of integration contributes to latency [30,31]. Repressive chromatin makes an important contribution to latency (reviewed in [32]). Limiting levels of cellular transcription factors present in resting $CD4^+$ T cells also make important contributions to latency, especially P-TEFb, a RNA Polymerase II (Pol II) transcription elongation factor involved in the viral Tat protein's function [33].

An active area of current HIV research is the development of small molecules, termed latency reversing agents or LRAs, which can selectively reactivate HIV, or “shock” HIV out of latency (Figure 1). This selective reactivation is an enormous challenge given that the Poll II transcriptional apparatus, which transcribes the HIV provirus, also transcribes all cellular protein coding genes. LRAs with ability to reactive HIV include histone deacetylase inhibitors (HDACi) [34,35], histone methyltransferase inhibitors [36], the anti-alcoholism drug disulfiram [37], protein kinase C (PKC) agonists [38-40], proteasome inhibitors [41], and Toll-like receptor 7 (TLR-7) agonist [42]. Of these LRAs, the PKC agonist, Ingenol 3,20-dibenzoate, and a TLR-7 agonist, have demonstrated the most significant effects on reactivation of HIV and SIV, respectively.

It has become clear that a single LRA which targets a single mechanism involved in latency is unlikely to be effective *in vivo*. Rather, multiple LRAs will likely be required to reactivate latent viruses *in vivo*, such as a combination of LRAs that target repressive chromatin and act as PKC agonists to up-regulate transcription factors such as NF-κB and P-TEFb [43]. Additionally, many latent replication-competent proviruses are refractory to reactivation [5], and it is likely that effective reactivation of latent HIV may require multiple courses of LRA treatment. As all the initial LRAs being tested were developed for other therapeutic purposes, they may have dose-limiting toxicities for uninfected cells either alone or in combination. Development of novel generations of LRAs for inducing HIV expression may lead to greater latency reversal with higher specificity and without increasing the potential for adverse effects.

Challenges to immune-mediated clearance of the latent reservoir

The failure of eradicating or even decreasing the size of HIV reservoirs by LRAs suggests that the reactivation of latent HIV does not leads to efficient activation of cytotoxic T celsl (CTLs) to kill infected cells. While it is of considerable interest to boost CTL responses against HIV in order to eliminate cells induced to express virus with a LRA, there are clear challenges to doing so. First, when HAART is initiated late after infection, CTL escape mutants dominate the latent viral reservoir [44]. Second, it is also becoming increasingly clear HIV may take advantage of immune-privileged sites such as B cell follicles of lymph nodes for persistence. Nonhuman primate studies with SIV have demonstrated that B cell follicles are inaccessible to virus-specific $CD8^+$ T cells [45], and that continuous viral replication occurs in $CD4^+$ follicular helper T cells of elite controller animals with effective SIV-specific $CD8^+$ T cells [46]. Thus, broad CTL responses will need to be induced, but there will also need to be a way to temporarily overcome critical immune regulatory mechanisms meant to prevent immunopathology in sites vital to developing adaptive immune responses. Finally, an additional potential problem identified is that some LRAs (namely, histone deacetylase inhibitors romidepsin and panobinostat) used to reactivate latent HIV may also impair CTL responses against HIV-infected target cells [47].

Adoptive T cell therapy to enhance elimination of infected cells

Treatment with LRAs should lead to active viral production from at least a portion of the reservoir of latently infected cells. However, LRAs alone have not led to significant decreases in $CD4^+$ T cell associated proviral DNA in HIV patients [7]. This suggests that the immune system has failed to kill the cells expressing viral antigens after stimulation by LRAs. Therefore, passively waiting for the development of HIV-specific CTL response after LRA treatment appears to be ineffective. It may be critical to develop an active T cell therapy approach for HIV [48]. A HIV patient's CTLs can be activated and expanded *ex vivo* with the patient's own antigen-presenting cells pulsed with dominant HIV epitopes. The activated CTLs can then be re-introduced back to the patient for adoptive T cell therapy in combination with LRA treatment (Figure 1). Although past studies did not demonstrate clearance of HIV infection by passive infusion of HIV Gag-specific $CD8^+$ T cells, new approaches for *ex vivo* expansion of broadly-specific CTLs targeting multiple viral antigens from HIV patients have been shown to kill HIV reactivated from the latent reservoir [49,50]. This may be more efficient for decreasing HIV viral loads by killing a significant portion of the reactivated viral reservoir. Alternatively, therapeutic vaccination may be necessary to boost HIV CTL responses of the patient prior to treatment with LRAs in order to reduce virally infected cells [51].

Passive immunotherapy with broadly neutralizing antibodies

A handful of potent broadly neutralizing antibodies (bNAbs) against HIV have been identified in HIV-infected individuals. Several recent studies indicate that passive administration of cocktails of bNAbs may be effective at lowering plasma viremia and proviral DNA in peripheral blood and tissues of SHIV infected rhesus macaques [52,53]. In one of the studies, Gag-specific T cell responses demonstrated improved functionality after

bNAb administration, and some animals showed prolonged virological control even after waning of the infused antibodies. Interestingly, newborn rhesus macaques treated with neutralizing antibodies during acute infection with SHIV appeared to be free of virus 6 months after exposure [54]. Finally, passive administration of bNAbs just prior to suppressive ART in SHIV-infected macaques resulted in a more rapid reduction in viremia and lower proviral DNA in lymph node $CD4^+$ T cells than controls [55]. One advantage of using antibodies over $CD8^+$ CTLs is they are not anatomically restricted from B cell follicles. Thus, passive immunotherapy with bNAbs may be a promising way to improve clearance of infected cells after latency reversal in the setting of HAART treated HIV patients (Figure 1).

Promoting death of infected memory $CD4^+$ T cells

As there may be challenges to immune mediated clearance of infected $CD4^+$ T cells and the cells appear to be resistant to dying after viral reactivation with a LRA, other strategies such as modulating cellular metabolism or cell survival pathways in order to enhance killing of HIV-infected cells after reactivation are attractive but underexplored strategies (Figure 1). Indeed, interfering with cellular metabolism alters both cell survival and virus production [56]. New studies also suggest that virus producing $CD4^+$ T cells may be protected from undergoing apoptosis via viral protease-mediated cleavage of procaspase 8 and binding of the peptide fragment (Casp8p41) to the mitochondrial proapoptotic protein BAK. Following reactivation of viral gene expression, infected $CD4^+$ T cells were more susceptible to the induction of cell death by a Bcl-2 antagonist [57]. Therefore, sensitizing latently infected cells with cell death inducing agents may help promote the eradication of latently infected $CD4^+$ T cells after treatment with LRAs.

Conclusions

As latently infected $CD4^+$ memory T cells appear to be relatively resistant to immune mediated and virus mediated cell killing after reactivation, it will be important to explore and identify new strategies to clear the persistent viral reservoir. Current LRAs are probably not effective in reactivating the entire HIV reservoir. Development of novel classes of LRAs and using them in combinations may be more effective for reversing latency and inducing the virus expressing cells to die. Use of adoptive T cell therapy and passive immunotherapy with broadly neutralizing antibodies in addition to LRAs may compensate for the inefficiencies of the immune system for better clearance of HIV reservoirs.

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Highlights

- Memory CD4+ T cells with latently integrated proviruses are a major barrier to curing HIV infection.
- Proviral integration within genes involved in cancer may contribute to clonal proliferation of latently infected CD4+ T cells.
- Challenges to clearing viral reservoirs within B cell follicles of lymph nodes.
- Immunotherapy shows promise for clearing HIV infection.
- Combination approaches may be necessary to cure HIV infection.

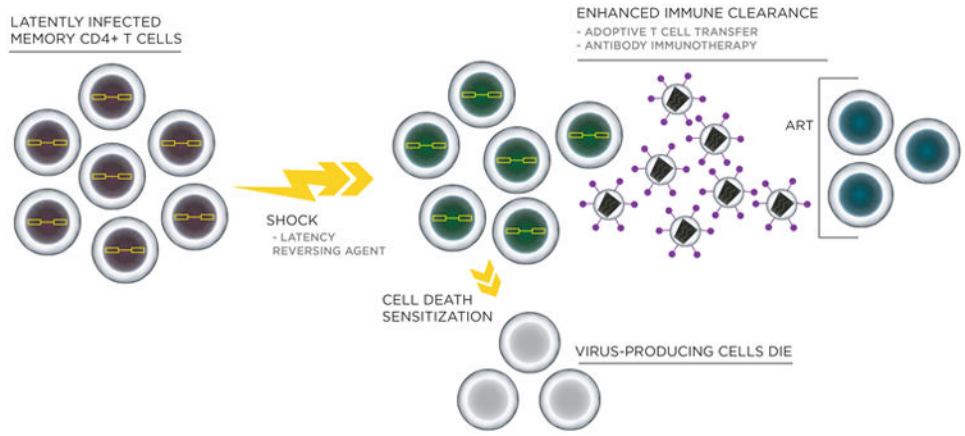

Figure 1.

Potential strategy for eradicating latently infected memory $CD4^+$ T cells. Although antiretroviral therapy (ART) effectively controls HIV replication, the virus persists as a latently integrated proviral form in resting memory $CD4^+$ T cells. As latency is an efficient immune evasion mechanism, it is of significant interest to devise ways to reactive virus expression in order for infected cells to be recognized by the host immune response and cleared, or for the cells to undergo virus-mediated cell death. However, neither process appears to readily occur following treatment with latency reversing agents. HIV cure strategies should therefore consider approaches to increase these activities. First, immune-mediated clearance of the latently infected $CD4^+$ T cells after reactivation of virus expression could be enhanced by adoptive T cell transfer of broadly specific anti-HIV CTLs and passive immunotherapy with broadly neutralizing antibodies (bNAbs). Second, virus-induced cell killing may be enhanced by sensitizing the cells with small molecule drugs targeting cell survival pathways.