REVIEW

Targeting the latent reservoir to achieve functional HIV cure
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Abstract

While highly active anti-retroviral therapy has greatly improved the lives of HIV-infected individuals, current treatments are unable to completely eradicate the virus. This is due to the presence of HIV latently infected cells which harbor transcriptionally silent HIV. Latent HIV does not replicate or produce viral proteins, thereby preventing efficient targeting by anti-retroviral drugs. Strategies to target the HIV latent reservoir include viral reactivation, enhancing host defense mechanisms, keeping latent HIV silent, and using gene therapy techniques to knock out or reactivate latent HIV. While research into each of these areas has yielded promising results, currently no one mechanism eradicates latent HIV. Instead, combinations of these approaches should be considered for a potential HIV functional cure.

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Introduction
In the twenty years since the implementation of highly active anti-retroviral therapy (HAART), the overall face of HIV as a global health issue has changed. HAART—composed of a cocktail of anti-retroviral drugs which target proteins expressed at different steps in the HIV replication cycle—can affect only cells that harbor actively replicating virus. HIV+ individuals are able to live fairly normal lives on maintenance HAART, with minimal side effects. Nevertheless, the effects of HIV infection continue to be evident in these suppressed individuals, who continue to suffer from a number of metabolic, immunologic, and neurologic co-morbidities. Thus, despite reducing plasma viremia below detection limits, the virus is not eliminated. There is evidence that low levels of replication occur in suppressed individuals, primarily in tissue reservoirs; however, this is not reflected in systemic plasma viremia in these individuals. HAART requires life-long administration. Following even brief treatment interruption, HIV rebounds rapidly from its reservoirs. Goals of the present research are to eliminate, suppress permanently, or render cells inhospitable to the hidden HIV in infected individuals.

Research efforts to understand and target HIV reservoirs have focused on four main categories outlined in this review (Figure 1): first, reactivation of latent HIV by capitalizing on the ability of host cellular activation signals and transcription factors (TFs) to ‘shock’ the virus out of hiding; second, killing of reactivated HIV by strengthening the immune system, which has been crippled by the infection; third, keeping latent reservoirs permanently suppressed; and, finally, targeting HIV and CD4+ T cells, which are the primary host cells for the virus, via new gene therapy approaches.

Shock
Chronic infection by HIV is characterized by severe depletion of CD4+ T cells and continuing inflammation, which contribute to HIV-associated co-morbidities. Continued exposure to inflammatory cytokines exhausts the immune system. It also elevates the expression of the receptors programmed death 1 (PD-1) and cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) on T cells. Blockade of these molecules is used as a treatment for solid tumors and could reinvigorate exhausted T cells in HIV+ patients. These individuals also produce elevated levels of inhibitory cytokines interleukin (IL)-10 and transforming growth factor–beta (TGF-β). Indeed, blocking IL-10 results in increased T cell activity in a hepatitis C infection model.

Growth factor therapy, including treatment with IL-2, -7, or -15, is being explored as a means to stimulate T cell recovery. IL-2 and IL-7 are important T cell growth and proliferation factors. Infusion with IL-2 and IL-7 results in enhanced T cell production and memory T cell proliferation. IL-15 enhances cytotoxic CD8+ T lymphocyte (CTL) and natural killer (NK) cell activity in vitro. Indeed, the IL-15 super-agonist ALT-803 is currently in preclinical trials.

Silence
Four research areas, which reactivate HIV (1. shock), eliminate HIV (2. kill), silence HIV (3. silence), or alter the immune system to resist HIV (4. gene therapy) should contribute to the functional or complete cure of HIV in infected individuals. Within each area are individual components of that therapy. They can be applied individually or in combinations, which should decrease their doses and deleterious effects. Most likely, there will be additional approaches in the future.
Latent HIV is primarily found in resting CD4+ T cells in the periphery. Resting cells have low levels of cellular TFs, which are also required for HIV replication, including NF-κB, P-TEFb, and CDK11 [20, 21]. Among the first examined latency reversing agents (LRAs) were histone deacetylase inhibitors (HDACis) and BET bromodomain inhibitors (BETis), which induce chromatin stress and induce the release of positive transcription elongation factor b (P-TEFb) from its repressive complex [22]. HDACis—such as panobinostat [23], romidepsin [24], SAHA [25], and valproic acid [26]—and BETis—such as JQ1 [27]—all reactivate HIV in cell line models of latency. However, they do not work in human primary resting infected T cells [28, 29] because they contain very low levels of necessary TFs [30, 31]. Thus, clinical trials with SAHA resulted in only a modest and transient reactivation of HIV [32], making it an impractical mono-therapy for HIV reactivation.

Since HDACis and BETis do not increase levels of required TFs, some activation of CD4+ T cells is required. Indeed, protein kinase C (PKC) agonists, such as prostratin [33] and bryostatin [34], and the MAPK agonist procyanidin [35, 36] can reactivate HIV in cell line models and primary CD4+ T cells. However, prostratin is toxic at therapeutic levels, leading to muscle pain, respiratory distress, and hypertension. Bryostatin, derived from a marine animal, Bugula neritina, not only has similar side effects but is also cost prohibitive to manufacture. Because of these limitations, a number of synthetic analogues of prostratin and bryostatin with reduced toxicity in vitro are being developed [37–39]. Ingenols, which are purified from Euphorbia plants, represent additional PKC agonists of interest. Native and chemically modified ingenols reactivate HIV in cell lines and primary T cells [40–42]. These PKC agonists also increase cellular levels of necessary TFs [43]. Thus, select MAPK and PKC agonists represent attractive candidates to reactivate latent HIV.

Combining several of these approaches has the greatest potential to purge the viral reservoir. Indeed, lower doses of a T cell activator and an LRA (HDACi or BETi) can be administered for increased potency and reduced pro-inflammatory responses [44–46]. Further understanding of HIV integration, transcription, and reactivation, as well as host cell behaviors, will inform optimal combinations of activators and LRAs.

**Kill**

Strategies to remove HIV by enhancing the killing by CTL and NK cells [47] or via broadly neutralizing antibodies (bNAb)s represent the second major field of research in HIV eradication. It is also important to investigate kill strategies in the context of the aforementioned shock therapies because many of the treatments proposed to reactivate latent HIV also dampen CTL function [48], which is already impaired in HIV+ individuals [11].

Using modified cytomegalovirus (CMV), a live vaccine expressing several simian immunodeficiency virus (SIV) antigens, was found to protect rhesus macaques against viral challenge [49–51]. Vaccinated animals initially appeared to be infected; however, they gained protection against SIV and showed enhanced effector T cell function against viral antigens.

Another approach involves bNAb s [52]. Following infection, anti-HIV antibodies are abundant in HIV+ patients; however, owing to the ability of the virus to mutate, the majority of them fail to eliminate the virus. bNAb s are the exception, in that they recognize many clades of HIV as well as escape mutants of the virus. In several studies, they not only neutralized virions released from activated CD4+ T cells from patients [53] but also reduced the viral rebound following HIV reactivation in a humanized mouse model [54]. However, even the most potent bNAb s are each only effective against a narrow subset of HIV clinical isolates, suggesting that effective bNAb approaches may require a combination of several bNAb s [55]. A second antibody approach utilizes bispecific antibodies, wherein one arm of the Fab portion of the antibody recognizes HIV envelope and the second arm recognizes CD3, making the cell vulnerable to CTL-mediated killing.

Finally, in an effort to achieve more effective killing, chimeric antigen receptors (CARs), which increase T cell receptor avidity and activation, are being explored. They can be engineered to recognize specific viral proteins; CARs against CD19, which is a B cell receptor, led to an astounding 90% remission rate in acute lymphoblastic leukemia [56, 57]. However, one caveat to CARs is that these cells are long-lived and can have substantial off-target effects.

**Silence**

The success of HAART has demonstrated that keeping the virus suppressed results in markedly healthier individuals. Resting infected cells do not produce HIV. Thus, these strategies rely on reducing T cell activation, which should also reduce the HIV-associated inflammation found in chronically infected individuals [6]. JAK and STAT molecules are important signaling molecules associated with many cytokine receptors. Ruxolitinib and tofacitinib, two JAK inhibitors that are approved for the treatment of rheumatoid arthritis and myelofibrosis, were tested against HIV, HIV2, and simian HIV (SHIV). They inhibited HIV reactivation [58]; and, furthermore, ruxolitinib attenuated encephalitis symptoms in infected humanized mice [59]. Cyclosporine A, an immunosuppressant used primarily to prevent transplant rejection [60], inhibits T cell proliferation by blocking IL-2 signaling in T cells [61]. Infected patients treated with cyclosporine A had some T cell recovery [62] but limited suppression of HIV replication [63–65].

The inhibitor didehydro-cortisatin A (dCA) acts via a suppressive mechanism that primarily targets HIV transcription. dCA binds to the basic domain in the HIV regulatory protein Tat, inhibits its interactions with the RNA response element TAR, and prevents its activation of HIV transcription [66]. dCA inhibits HIV reactivation in cell lines, primary cells, and peripheral blood mononuclear cells (PBMCs) from HAART-suppressed patients [67]. Furthermore, dCA may also contribute to continued HIV suppression by inhibiting inflammatory cytokine expression [68].

**Gene therapy**

Recently, a number of groups have taken advantage of cutting edge gene therapy approaches to HIV cure. However, as with any gene therapy approach, the barriers include delivery, specificity, off-target effects, costs, and ethical concerns.
The single case of successful HIV cure was achieved by the reconstitution of the patient’s immune system with donor bone marrow containing a natural mutation in the CCR5 HIV co-receptor. This patient was treated for acute leukemia with several courses of total lymphoid irradiation followed by two separate bone marrow transplants. Attempts to replicate this therapy used the Zn++ finger nuclease and more recently CRISPR/Cas9 targeting of CCR5 to induce the delta 32 mutation in patients’ own hematopoietic cells, which were then returned to the host. Since only mature cells were used, the effects of these manipulated cells were not permanent. Recent work using CRISPR/Cas9 to target the second HIV co-receptor, CXCR4, has also yielded promising results.

While HIV and SIV are highly related viruses, HIV cannot infect non-human primates, as their restriction factors block HIV infection more effectively than their human counterparts. Therefore, altering human restriction factors to behave like their simian counterparts represents an attractive strategy. One such factor is TRIM5. Of special interest is TRIM5 from owl monkeys, which is linked in frame to cyclophilin A, and this fusion protein blocks HIV. Using lentiviral vectors to deliver Trim-Cyp has blocked HIV effectively in cell lines and primary T cells. Additionally, it has been used successfully in a triple combination anti-HIV lentiviral vector approach in an infected humanized mouse model.

Recently, CRISPR/Cas9 technology has emerged as the most versatile and effective gene therapy approach. Using a DNA targeting strategy utilized by bacterial CRISPR, any number of specific guide RNAs can be loaded into the Cas9 protein to target specific areas of DNA for knock out or knock in of genes. Similarly, this technology has been used to knock out and reactivate latent HIV. Targeting various regions of the HIV LTR inactivated the virus in infected cell lines and prevented their reinfecction. However, viral target sequences can mutate, and HIV LTR-specific guide RNA can fail to recognize and target the mutant sequences, preventing long-term eradication by this method. To reactivate HIV, a defective Cas9 protein (dCas9) is used, which is fused to four copies of the herpes simplex VP16 activation domain (VP64) or a synergistic activation mediator (SAM) complex. Again, guide RNAs bring these dCas9 activators to the initiated transcription machinery. This targeting results in potent reactivation in latently infected cell lines.

Summary

Although HIV infection in the era of HAART has become a manageable chronic infection, problems with adherence to drug regimen, co-morbidities, and the emergence of drug resistance emphasize the need for continued research into HIV cure. Since the barrier to cure is the HIV reservoir, targeting this persistent virus is critical. The approaches detailed in this review represent a spectrum of the current research: however, eliminating the remaining 10^6 to 10^9 latently infected cells will require a combination of approaches. Mechanisms, such as HIV reactivation, will reveal hidden virus. However, the severely crippled immune system and further decreased CTL function indicate that it must be paired with the boosting of anti-viral host defenses. Likewise, keeping latent HIV in a suppressed state could keep HIV+ patients relatively healthy but less able to resist other infections and/or cancer. Using gene therapy to create a parallel immune system, where cells resist HIV infection, could complement all other approaches but is not scalable or affordable in resource-poor countries. While none of these approaches represent the eradication of HIV, combining several treatment modalities could bring us closer to a functional cure, where prolonged HAART-free and disease-free intervals would be achieved in infected patients.

Competing interests

The authors declare that they have no competing interests.

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