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HIV-specific CD8+ T cells and HIV eradication

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Introduction

HIV is an infection of the immune system that, despite induction of both humoral and cellular immune responses, is not eliminated. Animal models show that a stable reservoir of quiescent CD4+ T cells containing integrated provirus is created within days following transmission (1). Despite the induction of vigorous, HIV-specific CD8+ T cell responses that would be expected to eliminate infected cells (2–4), the immune system appears incapable of clearing this reservoir. This is at least partially attributable to the greatly reduced or absent viral antigen expression that occurs in these quiescent “latently” infected cells. Additionally, virus escape from CD8+ T cell recognition, CD8+ T cell dysfunction, and compartmentalization of both CD8+ T cells and viral reservoirs limit the efficacy of the naturally induced immune response to clear infection. Indeed, 35 years into the epidemic, there are no documented cases of immune-mediated clearance of established infection.

In the absence of effective CD8+ T cell–mediated viral clearance, combination antiretroviral therapy (cART) can effectively contain viral replication; however, like the adaptive immune response, cART does not eliminate infected quiescent cells, because the viral enzyme targets of the antiviral therapies are not required once the provirus has been integrated into the host genome.

The latent reservoir appears to have been eliminated and a cure achieved (5–7) in one bone marrow transplant recipient, in whom donor cells were homozygous for a 32-bp deletion in the HIV coreceptor CCR5, rendering the repopulating cells resistant to infection. The combination of conditioning regimen and graft-versus-host disease (GVHD) may have also contributed to the elimination of the reservoir and apparent cure. This case has mobilized intense efforts toward HIV eradication, ideally with less toxic interventions. Foremost are attempts to pharmacologically reactivate virus from latently infected cells using a variety of latency-reversing agents (LRAs). However, emerging data indicate that LRA-treated cells do not die by viral cytopathic effects, suggesting that eliminating them through engagement of HIV-specific CD8+ T cells will be required if this approach is to succeed (8, 9). For clearance to occur, the CD8+ T cell response will have to be more effective than it is in natural infection. Here, we discuss the prospects for the contribution of HIV-specific CD8+ T cells to elimination of the viral reservoir in the context of long-term cART. Short of viral eradication, we discuss the prospects for harnessing HIV-specific CD8+ T cells to contain rather than eradicate virus replication, effecting a functional cure as defined by sustained remission of viremia after cessation of therapy.

Antiviral efficacy of HIV-specific CD8+ T cells

Viruses are typically eliminated by virus-specific CD8+ T cells, which recognize processed viral proteins that are presented as a complex with an HLA class I molecule at the surface of an infected cell. Recognition through the T cell receptor (TCR) initiates a cascade of activation events, ultimately leading to the release of granzymes and perforin and killing of the infected cell, which can occur before infectious progeny virions are produced (10). Additionally, TCR activation leads to the release of a variety of cytokines including IFN-γ, TNF-α, macrophage inflammatory proteins 1α and 1β (MIP-1α and MIP-1β), and RANTES (CCL5), which have antiviral effects.

Numerous lines of evidence suggest that HIV-specific CD8+ T cells exert potent antiviral effects. The magnitude and rapidity of HIV-specific CD8+ T cell activation in hyperacute infection correlate inversely with the viral load set point (4), indicating that these cells mediate antiviral pressure during peak viremia (2, 3). Antiviral pressure is further indicated by rapid evolution of escape variants within targeted viral CD8+ T cell epitopes following acute infection (11, 12). In vitro models provide additional evidence for an antiviral effect, showing that these cells potently inhibit viral replication (10, 13). This is consistent with animal model data showing that depletion of CD8+ T cells following acute infection

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leads to high-level viremia that decreases as CD8+ T cells reappear (14). Genetic studies indicate that HLA class I alleles are associated with differences in set-point viremia (15, 16), modulated by the nature of viral peptide binding to the class I groove (16). Studies of viral fitness indicate that CD8+ T cell–induced mutations can diminish viral fitness, particularly those in epitopes restricted by protective HLA alleles such as B*27 and B*57, suggesting a persisting antiviral effect, even in cases of immune escape (17–19). Together, these studies indicate that CD8+ T cells are capable of potent antiviral function and provide a strong rationale for enlisting these responses in eradication strategies.

HIV latency under cART as a barrier to CD8+ T cell–mediated eradication

In the mid-1990s, several groups recognized that HIV could establish infection in resting CD4+ T cells in patients (20–24). As detectable virion production does not occur in these resting cells but can be induced by mitogens and other agents, these cells were defined as being “latently” infected. Longitudinal studies indicated that this reservoir was extremely stable under cART, with a half-life of 44 months (22). The ability of these cells to persist for years to decades without being killed by either viral cytopathic effects or immune effectors, such as CD8+ T cells, and then to re-seed systemic viremia is accepted as a primary mechanism by which infection persists despite effective cART. The expression of HIV antigens, which is either absent or low in these quiescent cells, is a prerequisite to their targeting and elimination by CD8+ T cells. To be effective at eradicating this reservoir, CD8+ T cells will likely need to be combined with LRAs, which induce antigen expression (see below).

While the role of latency in protecting cells from viral cytopathicity is clear, its status as an absolute barrier to cytotoxic T lymphocyte–mediated (CTL-mediated) killing is perhaps less self-evident. As T cells can detect even a single MHC-peptide complex on a cell surface (25), a remarkably strict state of latency would need to be maintained over years for CD8+ T cell killing of latently infected target cells to be absent in antiretroviral-treated (ARV-treated) subjects. While there are major defects in transcriptional initiation and elongation in resting CD4+ T cells (26–29), both unspliced and multiply spliced HIV transcripts can be detected in resting CD4+ T cells from HIV-infected individuals (29–34). The degree to which these transcripts result in the translation of HIV gene products may be limited by several factors, including retention in the nucleus (33), transcriptional interference, and “read-through” transcription (35). The studies described above support the possibility that low-level HIV antigen expression may occur in at least a subset of resting CD4+ T cells.

Currently, it is not possible to directly detect the minute amount of HIV antigen needed to trigger CD8+ T cells that might be expressed on the approximately 1 of 10^6 resting CD4+ T cells that typically harbor an inducible intact provirus in ex vivo samples. However, studies in primary cell latency models have revealed that some expression of HIV Gag does occur in this system (36, 37), suggesting that some cells are transcriptionally active. Given the multitude of factors limiting the potency of CD8+ T cells in untreated infection that restricts their ability to control viremia, latency may not fully explain the inability of CD8+ T cells to eradicate persistent reservoirs in ARV-treated subjects. Further study of the interaction between HIV-specific CD8+ T cells and latently infected cells is warranted, as is further consideration of the possibility that these cells may play an ongoing role in limiting the viral reservoir in ARV-treated subjects.

Effects of cART on CD8+ T cell responses

Suppression of HIV replication by cART leads to the reduction or elimination of antigen expression, thereby affecting the magnitude and breadth of effector CD8+ T cell responses that would be poised to kill virus-infected cells. Although cART often results in a transient rebound of detectable CD8+ T cell responses in individuals with advanced immunosuppression (38), in subjects with less advanced disease, CD8+ T cell responses decline rapidly in the first weeks of ARV initiation (39). Once viremia is fully suppressed, HIV-specific CD8+ T cell responses in the peripheral blood continue to decay with a t1/2 of 38.8 weeks for at least 2 years (40–43). The overall implications of cART-associated decay kinetics for HIV eradication strategies are not entirely clear without knowing the density of HIV-specific CD8+ T cells that would be
required to clear viral reservoirs at tissue sites of viral reactivation and how this relates to frequencies of HIV-specific CD8+ T cells in the peripheral blood. In one sense, it is encouraging that CD8+ HIV-specific T cell responses are readily detectable in the majority of individuals, even after several years of ARV therapy, and that cART reduces the frequency of proapoptotic HIV-specific CD8+ T cells (44). However, CD8+ T cell responses may need to be activated to an effector phenotype and/or expanded in frequency in order to effectively contribute to eradication.

In addition to allowing for the contraction of HIV-specific CD8+ T cell responses, the reduction or elimination of HIV antigen in ARV-treated subjects leads to alterations in the phenotypic and functional profiles of the remaining cell populations. In untreated HIV infection, as in other chronic viral infections, persistent exposure to antigens leads to the progressive dysfunction of virus-specific CD8+ and CD4+ T cells, a phenomenon termed “T cell exhaustion” (reviewed in refs. 45, 46). In the early stages of exhaustion, T cells exhibit impaired proliferation in response to antigen and reduced polyfunctionality (the ability to produce multiple cytokines) (47). This is followed at late stages by apoptosis of HIV-specific CD8+ T cells (48). This exhausted state is associated with the upregulation of multiple activation and coinhibitory molecules (see below) (49–55). Several studies have demonstrated that prolonged ARV therapy results in some restoration of polyfunctionality and at least partial downregulation of activation and exhaustion markers (49–52, 56–60). It is tempting to speculate that, despite being reduced in numbers, the HIV-specific CD8+ T cells that remain in ARV-treated subjects may be more functional, less restrained by coinhibition, and less proapoptotic. Thus, on a per-cell basis, the remaining cells may be more able to eliminate reactivated HIV-infected cells than pre-ARV CD8+ T cells.

While it may be true that on cART, HIV-specific CD8+ T cells exhibit improved function, our knowledge of the functional and phenotypic features of HIV-specific CD8+ T cells with the greatest potential for eradicating reservoirs is inadequate to draw conclusions. Eradication may require only relatively rare and diffuse encounters between CD8+ T cells and residual infected cells with limited capacity to re-seed themselves, suggesting that the most desirable effectors will be highly specialized for cytotoxicity, even at the expense of proliferative capability or the ability to produce cytokines and chemokines to recruit or support other cells. Such considerations may have implications for the timing of therapies aimed at disrupting latency, whereby CD8+ T cells may be either gradually improving or regressing in their potential to eliminate exposed reservoirs.

**Additional limits of HIV-specific CD8+ T cell efficacy in HIV infection**

Despite evidence of potent antiviral function of HIV-specific CD8+ T cells, these cells are unable to fully clear infection, with plasma viremia persisting in most untreated infected persons. Under the best of circumstances, a détente is reached, in which plasma viral load is maintained at undetectable levels in plasma, although evidence indicates that tissue replication continues to occur in the vast majority of so-called “elite controllers” (reviewed in ref. 61). Additional barriers to CTL-mediated eradication are discussed below and summarized in Figure 1.
responses, which exert only weak pressure (80, 81). While epitope presentation kinetics may play a role in this phenomenon, efforts to further define what distinguishes the most effective CD8+ T cell responses in this setting remain a highly active area of research. In the meantime, the evidence discussed above provides guidance for the design of immunotherapeutic strategies, such as preferentially targeting the Gag protein, that are aimed at controlling viremia in untreated infection.

The features that define an effective CD8+ T cell response in the context of reservoir elimination are likely distinct from those needed to control active viremia and should also be considered in the context of therapeutic immunization. In the setting of active viremia, it is critical that CD8+ T cells target a conserved epitope, such that either escape cannot occur, or CTL-induced mutations in vulnerable regions reduce viral fitness (78, 79). In ARV-suppressed patients, the lack of viral replication negates the issue of ongoing viral escape, though sequence diversity in the existing reservoir is an important consideration. In unsuppressed patients, it is critical for CD8+ T cells to be able to recognize infected cells quickly, before virus can be produced (82–85). In ARV-treated subjects, rapid killing may not be important, provided that transmission of infection to other cells is suppressed by ARVs. High-avidity CD8+ T cells may also be important for eradication, whereby LRAs may induce only low-level antigen expression. The above-mentioned points are speculation provided to illustrate a gap in knowledge that must be addressed to most effectively harness CD8+ T cells for HIV eradication.

**CD8+ T cell compartmentalization and the viral reservoir.** Compartmentalization likely limits the ability of HIV-specific CD8+ T cells to eliminate infected cells. Pioneering studies in HIV-infected subjects demonstrated that HIV-specific CD8+ T cells were largely excluded from lymph node follicles, and CD4+ T cells in the lymphoid follicle were, on average, 31-fold more likely to be productively infected as compared with those in the paracortex (86). Studies performed in the SIV-infected rhesus macaque model have confirmed and extended these observations. The SIV model of elite control is driven by highly effective virus-specific CD8+ T cells that are able to recognize and eliminate at least a subset of infected cells systemically and in the lymph node paracortex without the aid of LRAs, with compartmentalization constituting a primary barrier to eradication (87, 88).

While the lymphoid follicles provide a clear-cut example of HIV persistence facilitated by the lack of HIV-specific CD8+ T cell access, similar scenarios may also exist in immune-privileged sites such as the testicles and CNS (89–92). Additionally, immune compartmentalization may contribute to HIV persistence in more subtle ways. CD4+ and CD8+ tissue-resident memory T cells (Trm) that are clonally expanded in tissue sites and do not readily circulate have recently been identified (93-100). The contributions of infected CD4+ Trm to HIV persistence remain unknown, as does the potential for CD8+ Trm to contribute to eradication by these tissue sites. One potential consequence is that infected CD4+ Trm that are localized in sites with restricted CD8+ T cell access will not expose themselves to killing by periodic egress. A second consequence is that infected CD4+ Trm in a given site may harbor escape mutations in CD8+ T cell epitopes that are not well represented in the peripheral blood. On an optimistic note, strategies to mobilize CD8+ Trm in tissues may have the potential to contribute to eradication, particularly if these cells have desirable specificities or functional profiles that are not represented in the circulation.

**CTL-mediated approaches for eradication of HIV infection.** There are multiple challenges to harnessing CD8+ T cells to eradicate a reservoir, as outlined above and as evidenced by the inability of these cells to eradicate the reservoir in treated or untreated natural infection. Nevertheless, there are a number of compelling strategies that are worthy of pursuit.

**Shock and kill.** A promising eradication strategy involves combining LRAs, such as histone deacetylase inhibitors (HDACIs), cytokines, TLR agonists, or others, with CD8+ T cells (or other immune effectors) in order to induce antigen expression from quiescent cells and then eliminate these exposed targets (101). When combined with expanded HIV-specific CD8+ T cell lines, this approach has been shown to drive the elimination of infected cells from a primary cell model of latency and from patient samples in vitro (8, 102). Despite evidence that the administration of certain HDACIs disrupted HIV latency in patients, none of these studies revealed detectable reservoir depletion. One potential explanation for this finding could be that HDACIs impaired CD8+ T cell function in vivo, thereby interfering with the ability of these cells to eliminate exposed target cells. Both panobinostat and romidepsin have been shown to interfere with multiple CD8+ T cell functions, including elimination of HIV-infected cells, when tested in vitro at pharmacologically relevant concentrations (103). Additionally, HDACIs exhibit immunosuppressive activities in animal models of GVHD, experimental autoimmune encephalomyelitis, and other diseases for which they may be of therapeutic benefit (104-106). Encouragingly, ex vivo assessment of CD8+ T cell responses in clinical trials involving HIV-infected participants has thus far shown a lack of detectable impairment following the administration of panobinostat or vorinostat, though increases in CD4+ Treg frequencies were observed (32, 107). The question remains as to whether the degrees of latency reversal observed in these trials were sufficient to expose latently infected cells to immune recognition. If not, the potential to negatively impact CD8+ T cell function with higher dosing regimens may define an upper limit on the therapeutic windows of these agents. Moreover, as activation through TCR stimulation sensitizes T cells to romidepsin and panobinostat toxicity in vitro (103), it is possible that CD8+ T cells that have been recently boosted by therapeutic vaccination may be preferentially killed by subsequent HDACI treatment. Moving forward, it will be important to continue to assess the potential impact of LRAs on the immune effectors with which they will need to work in concert in order to either mitigate potential interference or capitalize on potential enhancements of immune function. It will also likely be important to combine LRAs with strategies to address the other limitations of CD8+ T cells described above, including epitope escape and the diminished magnitude of responses observed in cART-treated subjects.

**Therapeutic immunization.** Peripheral blood mononuclear cells (PBMCs) from cART-treated subjects exhibit fairly weak ex vivo CTL-mediated killing of HIV-infected cells. Moreover,
cART-associated reductions in viremia skew cells toward a memory phenotype. CD8^+ T cell effector activity can be substantially enhanced by short-term expansion with HIV antigens (8, 102, 108). Successful in vitro demonstrations of the shock-and-kill concept have utilized such expanded HIV-specific CD8^+ T cell lines (8, 102) and can be replicated in vivo by administering therapeutic vaccines aimed at boosting cellular immunity prior to administering LRAs (reviewed in ref. 109). The issue of immune escape presents an unfortunate complexity, in which — with the exception of subjects whose cART was initiated during their primary infection — immunodominant CD8^+ T cell responses that might be preferentially boosted by therapeutic immunization are largely targeted against escaped epitopes and are therefore of no utility to HIV eradication (9). Therapeutic immunization to enhance cure efforts will likely require expanding the breadth of responses to include subdominant epitopes that have not already escaped. A related approach would involve the de novo priming of novel epitope specificity targeting CD8^+ T cell responses that are both critical for maintaining effective CD8^+ T cell function and able to reverse some of the functional defects acquired during prolonged viral exposure (113).

**Cell therapy.** Ex vivo expansion and reinfusion of antigen-specific T cells has shown tremendous promise as a safe and effective means of augmenting antiviral immunity to CMV and EBV and as a therapeutic modality for cancer (114–116). A limited number of attempts have been made to translate this approach to HIV (117–121). The sole study that infused oligoclonal-expanded natural T cells into HIV-infected patients was performed in the early days of ARV therapy, when suppression was poor and showed a trend toward increased CD4 counts and decreased viremia in the absence of toxicity (120). The strategy of ex vivo expansion and reinfusion of virus-specific CTLs offers a superior measure of control over epitope specificity and functional characteristics that is particularly well suited to focusing responses against nonescaped epitopes (reviewed in refs. 122, 123). Cell therapy additionally offers the intriguing possibility of addressing issues related to compartmentalization, as particular homing profiles can be imprinted on CD8^+ T cells by ex vivo culture conditions. For example, expanding T cells in the presence of retinoic acid results in subsequent homing of these T cells to the gut (124). T cell therapy involving the expansion of natural virus-specific responses has an excellent safety record (125), can be performed for approximately $6,000 per patient (126), and can establish populations of long-lived memory cells.

As an alternative to expanding natural HIV-specific T cell responses, cell therapy products can consist of T cells that have been redirected to recognize HIV-infected cells by genetic modification. This can be achieved by transducing cells with either transgenic HIV-specific TCRs or chimeric antigen receptors (CARs). These approaches offer several potential advantages, including the possibility of engineering high-avidity TCRs that may have enhanced abilities to detect viral reservoirs (127) and freedom from MHC-I restriction in the case of CAR T cells (128). However, unlike the expansion of naturally occurring HIV-specific T cell populations, these approaches must also address safety considerations regarding the possibility of unintentional targeting of self-antigens.

**Coinhibitory blockade.** Coinhibitory receptors, including PD-1, TIM-3, CD160, 2B4, LAG-3, and CTLA-4, play a critical role in the maintenance of exhaustion (49–55). Blockade of these receptors — either alone or in combination — has enhanced T cell function in vitro and viral control in multiple animal models (49–55, 129–132), providing a rationale for testing coinhibitory pathway blockade as an immunotherapeutic strategy in HIV infection. Additional enthusiasm for this approach can be drawn from advances in cancer immunotherapy, in which Abs that block the PD-1 and CTLA-4 pathways have been highly successful and are considered breakthrough drugs in the treatment of solid tumors (133). While treatment with CART results in some level of downregulation of coinhibitory receptor levels in the majority of HIV-infected subjects, these levels do not fully normalize in peripheral blood T cells, and persistent upregulation may be more pronounced in lymphoid tissues (49–54, 56, 57, 134). Thus, therapeutic blockade of coinhibitory pathways represents a promising approach to enhancing the abilities of CD8^+ T cells to clear persistent viral reservoirs.
Additional immunotherapeutics. As an alternative or adjunct to blocking inhibitory pathways, CD8+ T cell function can be enhanced by the provision of cytokines or other immunostimulatory agents. IL-15 agonists are of particular interest in this regard, having been shown to enhance CD8+ T cell and NK cell activity in a number of preclinical models (135–139), and the IL-15 superagonist ALT-803 is moving into a clinical trial in CART-treated HIV-infected subjects (ClinicalTrials.gov identifier: NCT02191098). Other immunostimulatory agents include TLR-2 agonists, which reverse CD8+ T cell exhaustion and enhance both tumor- and pathogen-specific T cell responses in vivo (140–142), and agonistic Abs against 41BB or CD40 (143–145), among others.

Conclusions
The cellular immune response has evolved to specifically target and eliminate intracellular pathogens, primarily viruses. In this Review, we have taken the pragmatic approach of mainly focusing on the barriers to the effective targeting of CD8+ T cells against the viral reservoir that persists in the setting of cART (Figure 2). Faced with the challenge of achieving HIV eradication, we do, however, draw considerable inspiration from the other precedents that illustrate the power of a cellular immune response that has been properly unleashed. Several recent examples of this come from the oncology setting, in which both checkpoint blockade inhibition (146) and T cell–based therapies have resulted in substantial clinical benefits (128, 147). These successes include some dramatic cases, in which, for example, a single dose of anti–CTLA-4 Ab resulted in the eradication of a large tumor mass (148). An additional example emanates from the SIV rhesus macaque model of HIV infection, in which a subset of animals that received rhesus CMV–vectored SIV vaccine went on to have seemingly eradicated nascent infections (149). While clearance in this model has only been demonstrated when the vaccine was given prophylactically, these studies provide a critical proof of principle for immune-mediated eradication of a lentiviral infection. With recent advances in cell therapy and vaccine platforms and growing clinical experience with immunotherapeutics, we have multiple tools available to attempt to overcome the factors limiting CD8+ T cell efficacy in preclinical models and in the clinic. A particular challenge may be the need to address multiple barriers in parallel in order to achieve a measurable benefit. For example, boosting the total magnitude of the CD8+ T cell response and enhancing function on a per-cell basis may be ineffective without a component that targets these responses against nontargeted epitopes.

This Review has focused on the prospects for harnessing CD8+ T cell responses to contribute to the eradication of infection, i.e., to achieve a “sterilizing cure.” An equally important strategy proposes to enlist cellular immune responses to exert long-term control of viremia without eliminating all reservoirs, i.e., to achieve a “functional cure.” Unlike sterilizing cures, functional cures have precedents in natural infection of patients who are elite controllers and in viremic controllers who maintain low levels of viremia in the absence of cART therapy. An additional precedent comes from the phenomenon of post-treatment control, as observed in the VISCONTI (giro-immunologic sustained control after treatment interruption) study cohort, in which subjects who were treated with cART during primary HIV infection showed a disproportionately high likelihood of maintaining low levels of viremia upon stopping cART (150). Many of the challenges in harnessing CD8+ T cells to achieve functional cures overlap with those involved in sterilizing cures, such as mitigating T cell exhaustion. There are, however, distinctions. For example, while achieving sterilizing cures will only have to address a fixed level of viral sequence diversity (established before initiation of cART), functional cures will have to address some level of ongoing viral evolution. Pursuing both of these related objectives in parallel will allow for cross-fertilization of lessons learned and maximize the potential for novel therapeutics that will improve the lives of people living with HIV/AIDS.

Finally, while the optimization of various arms of the immune system needs to be considered separately, we stress the importance of developing combination therapies that bring together both the cellular and humoral arms of adaptive immunity with innate immune mechanisms in order to overcome the tenacity of a virus that manages to persist for decades, even in an environment rendered inhospitable to its replication by cART.

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