■ BENCH TO BEDSIDE

Targeting reservoirs to clear and cure

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HIV-1 vaccine development efforts have recently been focused on neutralizing antibodies, with the assumption that it is essential to neutralize free virus particles and prevent the initial infection of host cells. However, once stable reservoirs of infected cells are established, clearing the infection is much more difficult and will probably depend on other immune mechanisms. Recent experimental studies in the simian immunodeficiency virus (SIV) model of HIV-1 infection have provided evidence for immunologic clearance of an established SIV infection by a vaccine-induced T cell response[1]. The findings showing SIV clearance have important implications for vaccination and also come at a time when there is a resurgence of interest in the possibility of curing HIV-1 infection. Although antiretroviral therapy (ART) can largely or completely arrest viral replication[2], the stable latent reservoir of HIV-1 in resting memory CD4$^+$ T cells prevents eradication of the infection with ART alone[3], and viremia generally rebounds when treatment is stopped. The renewed optimism about curing HIV-1 infection is due in large part to two remarkable clinical cases in which a cure appears to have been achieved[4,5]. These recent experimental results and clinical studies shed light on the search for a general cure for HIV-1 infection and support the idea that vaccine-induced T cell responses may actually be more effective than early treatment in preventing the establishment of the latent reservoir.

Picker and his colleagues have previously described a cytomegalovirus (CMV)-based SIV vaccine that induces and maintains high levels of effector memory T cells at diverse tissue sites[6]. This vaccine confers the ability to control a pathogenic SIV infection upon ~50% of vaccinated macaques[6]. The reason that only half of the animals experienced this beneficial effect remains unclear; however, in those animals, the initial burst of viral replication was rapidly suppressed. Thereafter, the animals that controlled infection experienced only occasional 'blips' of detectable viremia, which declined in frequency over time. The efficacy of this vaccine derives in part from its ability to induce effector T cells that recognize multiple diverse epitopes in all the SIV proteins found in the vaccine, sometimes in ways that violate the usual patterns of antigen presentation by major histocompatibility complex molecules[7].

In the most recent study, Picker and his colleagues showed that a subset of vaccinated animals—after being unequivocally infected by an SIV challenge—seems to clear the infection after several months, as assessed by the absence of detectable viral RNA and DNA and by the failure of adoptive transfer of 6×10^7 leukocytes from these animals to produce infection in naive recipients[1]. Although very rarely, some patients with HIV-1 infection control viral replication through as yet incompletely understood immunologic mechanisms[8], but the complete clearance of an established SIV or HIV-1 infection through immunologic mechanisms, as shown in these vaccinated animals, is unprecedented. The authors raise the possibility that persistent effector memory T cell responses to HIV-1, maintained by new CMV-based therapeutic immunization strategies, might contain and eventually eliminate viral reservoirs in patients on ART or prevent rebound viremia in patients who stop treatment[1].

In considering possible cases of HIV-1 cure, a crucial question is whether a stable viral reservoir in resting memory CD4$^+$ T cells has been established. Typically, the reservoir is established in the first few weeks of infection, when levels of viral replication are high and immune responses to the virus are developing[9]. Treatment during acute infection generally fails to prevent the establishment of the reservoir, although it may result in a smaller pool of latently infected cells; whether very early treatment (within days of exposure) can prevent the establishment of the latent reservoir is not yet clear. Established reservoirs are a formidable barrier to cure. The only person with an established reservoir who has been cured is a patient with leukemia who received a bone marrow transplant from a donor homozygous for a deletion in the HIV-1 co-receptor CCR5 (ref. 4). The preparative regimen of chemotherapy and irradiation, along with the ensuing graft-versus-host disease, may have eliminated almost all host-derived cells of hematopoietic origin, including resting CD4$^+$ T cells harboring latent HIV-1. Even if a few latently infected cells remain in this individual, they have not rekindled the infection because almost all susceptible cells in this patient are now of donor origin and therefore resistant to infection. Two other HIV-1–infected individuals received bone marrow transplants from donors with wild-type CCR5 genotypes[10] while receiving ART to protect the donor cells from infection. No virus was detected in their plasma for several months after they stopped treatment, but there was eventually a sudden and rapid increase in viremia, probably because of the activation of one of the small number of latently infected cells not eliminated during the transplant. These results highlight the difficulty of clearing viral reservoirs.

In patients who do not require a bone marrow transplant for malignancy, other approaches will clearly be required to eliminate the latent reservoir. Current efforts are focused on identifying agents that will induce virus gene expression in latently infected cells, with the hope that these cells will subsequently be eliminated by viral cytopathic effects or by virus-specific cytolytic T lymphocytes (CTLs)[11].

There are rare situations in which the latent reservoir is not established. Because the generation of memory T cells does not start until after birth, very early treatment of infants infected *in utero* may allow cure, which is illustrated by the case of the 'Mississippi baby', who was treated with ART within 30 hours of delivery[5]. ART was stopped approximately 18 months later, but the child has remained aviremic for more than 1 year. It is interesting to consider that in the absence of a stable reservoir, HIV-1 infection is curable with the powerful ART regimens now available. Similarly, hepatitis C virus, which does not establish a latent reservoir, can now be readily eradicated with direct-acting antiviral drugs[12].

Given the problem posed by the latent reservoir, how can the clearance of SIV in vaccinated animals be understood? SIV establishes a latent reservoir that is very similar to that seen in HIV-1 infection[13]. In both infections, the frequency of latently infected resting CD4$^+$ T cells is very low (1 in 1×10^6 cells), suggesting most infected cells die before reverting back to a resting memory state in which latency can be established (**Fig. 1**). Decay dynamics suggest

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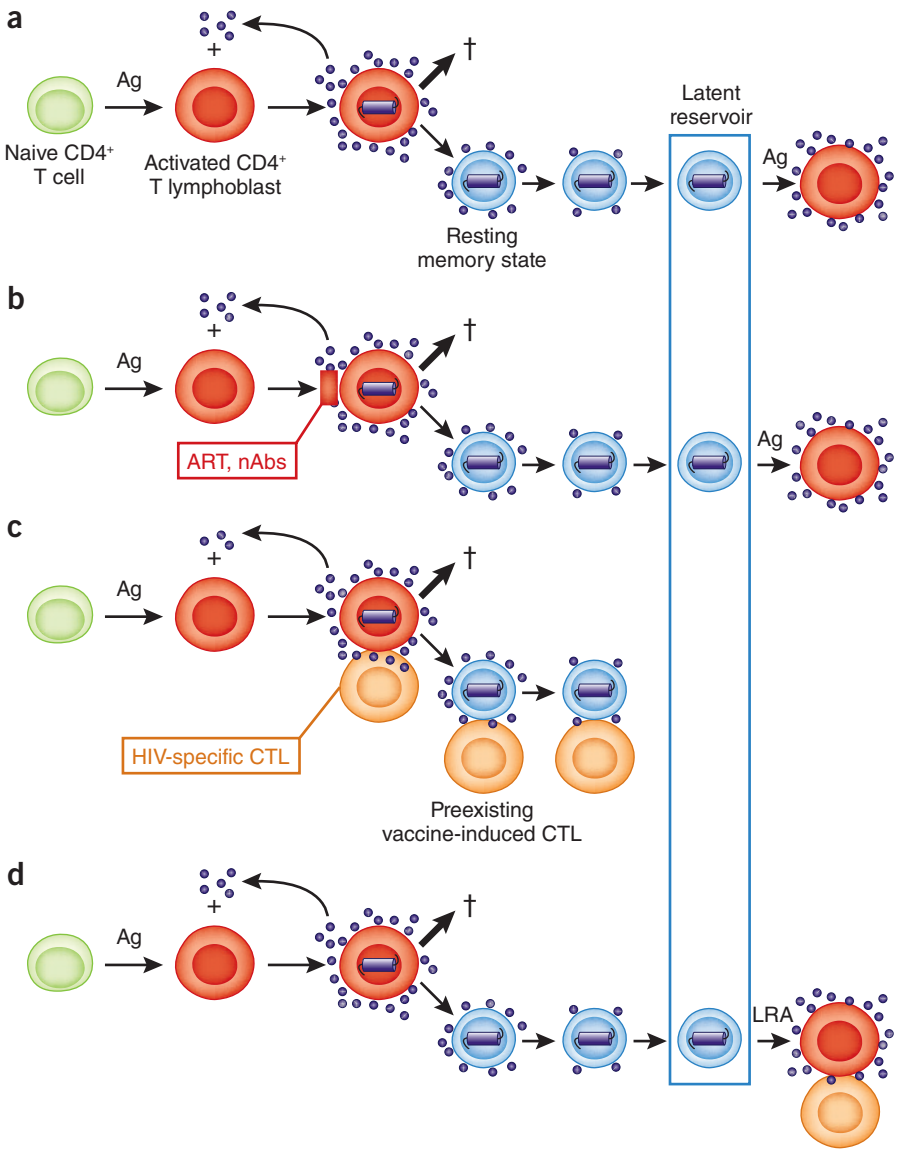

Figure 1 Preventing or eliminating HIV-1 reservoirs to cure infection. (**a**) Cell dynamics in untreated HIV-1 infection. Naive CD4+ T cells interact with antigen (Ag) and become activated CD4+ T lymphoblasts, which are highly susceptible to infection by HIV-1 (purple dots). Most productively infected cells die rapidly (†), but occasionally cells survive and slowly transition to a resting memory state. In fully quiescent cells, HIV-1 gene expression is turned off, generating a latent reservoir. Cells in the reservoir can be activated by antigen and begin to produce virus again. (**b**) ART and neutralizing antibodies both stop new cells from becoming infected. However, cells that have already been infected can still enter the latent reservoir. (**c**) Preexisting vaccine-induced CTLs can lyse infected cells before they can transition to a state of latent infection. (**d**) HIV-1–specific CTLs can facilitate the purging of the latent reservoir by lysing cells in which virus gene expression has been upregulated by latency-reversing agents (LRAs).

that the half-life of the cells that produce most of the plasma virus is very short, only 1 day[14], whereas a second population of productively infected cells, which may include CD4+ T cells in a low state of activation, turns over with a half-life of 2 weeks[14]. Some of these cells may survive and revert back to a resting state that is nonpermissive for viral gene expression and that allows for viral latency. During the considerable time required for this transition (weeks), the cells remain susceptible to lysis by virus-specific CTLs; thus, in vaccinated individuals, the preexisting effector memory CTL response can clear infected cells before latency is established (**Fig. 1**). In contrast, ART, which rapidly stops new infection of susceptible cells, has no effect on the fate of cells that are already infected. Because it takes some time for *de novo* T cell responses to develop, treatment with ART has not yet led to clearance except in the unique situation mentioned above, although it can largely halt new infection events. Thus, there is an important difference between vaccine-induced clearance and early ART with respect to the fate of infected cells.

Although the clearance of SIV infection in the vaccinated animals may not be interpreted as a cure because a stable reservoir was never generated in these animals, this study highlights the possibility of controlling the infection and clearing infected cells before a stable reservoir is established with an appropriately primed CTL response and suggests that a CTL-based vaccine could be effective. The disappointing results seen in the clinical trials of CTL-based vaccines to date have focused attention on neutralizing antibodies, which in principle can prevent any host cells from becoming infected.

However, Picker and his colleagues have now shown that viral clearance can occur even after disseminated infection in animals with strong vaccine-induced T cell responses[1]. The CMV-based vaccines do not elicit neutralizing antibodies[6]. The ability of vaccine-induced CTLs to lyse infected cells before latency can be established could be an important component of a successful AIDS vaccine and, when combined with vaccine strategies that induce neutralizing antibodies, could provide an additional layer of protection should some virus particles escape neutralization and initiate infection of host cells. With regard to curing patients with established HIV-1 infection, it will probably be necessary to use pharmacologic approaches to reverse latency so that the infected cells will begin to produce viral proteins and become susceptible to lysis by virus-specific CTLs. However, recent evidence suggests that the CTL response in most patients on ART is not effective in eliminating infected cells without boosting[15]. Whether CMV-based vaccine vectors can be safely used for this purpose is not clear, but they certainly deserve consideration.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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