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Perspective

Latent reservoirs of HIV: Obstacles to the eradication of virus

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The use of highly active antiretroviral therapy (HAART) in the treatment of HIV-1-infected individuals has provided a considerable amount of information regarding the dynamics of viral replication and has resulted in enormous advances in HIV therapeutics. The profound suppression of plasma viremia in HIV-infected individuals receiving HAART has resulted in a highly beneficial clinical effect and a dramatic decrease in the death rate attributable to AIDS. Nonetheless, the persistence of reservoirs of HIV, including latently infected, resting CD4$^+$ T cells that can give rise to infectious HIV upon stimulation *in vitro*, has posed a sobering challenge to the long-term control or eradication of HIV in infected individuals receiving HAART. Although a recent study has demonstrated that the size of the pool of latently infected, resting CD4$^+$ T cells can be markedly diminished with intermittent interleukin (IL)-2 and continuous HAART, complete eradication of HIV in infected individuals remains extremely problematic. Here, we discuss recent developments in studies of the latent reservoir of HIV in patients receiving HAART and implications for the long-term treatment of infected individuals and eradication of the infection.

With the advent of highly active antiretroviral therapy (HAART)—a treatment regimen consisting of a combination of at least three antiretroviral drugs and usually including at least one drug of the protease inhibitor class—for HIV-infected individuals, a greater degree of control of viral replication is now possible. In this regard, the widespread use of HAART has dramatically changed the clinical course of infection in many infected individuals and led to a substantial decline in the incidence of acquired immunodeficiency syndrome (AIDS) and AIDS-related mortality in the United States (1–5) and other developed countries (6–9). Furthermore, the study by Perelson *et al*. generated considerable optimism that HIV can be eradicated in infected individuals receiving HAART (10). This optimism was largely based on mathematical modeling of the kinetics of viral decay in the plasma of infected individuals shortly after initiation of HAART. However, the validity of this projection was predicated on the assumptions that viral replication is completely suppressed throughout that period of time and that no other unrecognized viral reservoirs with longer half-lives exist in infected individuals who are receiving HAART (10). Unfortunately, these assumptions have proven to be incorrect, and, thus, real concerns have arisen about whether complete eradication of HIV in infected individuals will ever be achievable using current HAART regimens (11–16). One of the most discouraging aspects of the feasibility of complete eradication of HIV from an infected individual is the persistence of latently infected, resting CD4$^+$ T cells carrying replication-competent HIV. These cells are likely a major reservoir for HIV, particularly when active virus replication is suppressed by HAART. In late 1997, three groups demonstrated independently that a pool of cells from which virus could be isolated persists in essentially all infected individuals tested who were receiving HAART for considerable periods of time (up to 3 years) and in whom plasma viremia was suppressed below levels of detection by the most sensitive assays (11–13). Pursuant to this sobering observation, intensive interest has been generated in the delineation of the nature of this viral reservoir, the process of its establishment, and the mechanisms of its persistence, as well as in strategies aimed at its containment and/or elimination.

Identification and Characterization of the Latent Reservoir of HIV in Resting CD4$^+$ T Cells

The presence of latently infected, resting CD4$^+$ T cells was initially demonstrated a few years before the wide-spread use of HAART in treating HIV-infected individuals (17). At that time, the understanding of viral latency was largely limited to the concept of preintegration latency (18, 19). HIV may enter resting CD4$^+$ T cells, at which point a limited degree of reverse transcription of the HIV genome may occur in these cells (18, 19). This period of preintegration latency may last hours to days; in the absence of an activation signal, proviral DNA loses its capacity to initiate a productive infection (18, 19). If these cells become activated, however, reverse transcription proceeds to completion, followed by nuclear translocation and integration of proviral DNA into cellular DNA (18–20). Using rigorous cell purification methods and a selective PCR technique that detects only integrated forms of HIV DNA, it has been shown that $<0.1\%$ of resting CD4$^+$ T cells carry integrated provirus (postintegration latency). In this form, the DNA that has been reverse transcribed from the genomic RNA of the virus is integrated into the cellular DNA and can remain transcriptionally silent. Among these resting CD4$^+$ T cells, only a fraction carry replication-competent HIV in infected individuals who are untreated or treated with a less than optimal regimen of one or two antiretroviral drugs (17, 21). Shortly after it was established that such a population of latently infected cells exists and may serve as a long-term viral reservoir in infected individuals, this initial observation was extended to patients who were receiving HAART. Despite a considerable amount of optimism in 1996 and 1997 regarding the possibility of the eradication of HIV, it was quickly shown that the latent HIV reservoir persists in essentially all infected individuals who were receiving HAART and in whom plasma viremia was suppressed below levels of detectability (11–13). In addition, we conducted a series of studies aimed at determining the time of establishment of the latent HIV reservoir and the factors involved in the induction of viral replication in this pool of cells. In this regard, we asked whether institution of HAART early during primary HIV infection could prevent the establishment of the pool of latently infected, resting CD4$^+$ T cells. First, we demonstrated that initiation of HAART in infected individuals as early as 10 days after the onset of symptoms of primary HIV infection did not prevent generation of latently infected, resting CD4$^+$ T cells (22). These findings underscore the rapidity with which latent reservoirs are established in primary HIV infection and indicate that it

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is highly unlikely that early treatment during primary infection can prevent establishment of a pool of latently infected, resting $CD4^+$ T cells if treatment is initiated after a substantial burst of plasma viremia has occurred and virus has been disseminated throughout the body (22). Second, we studied the role of activating stimuli, particularly cytokines, in the induction of virus expression from latently infected, resting $CD4^+$ T cells. We demonstrated that the combination of cytokines, particularly IL-2, IL-6, and TNF-α, is a potent inducer of viral replication in this pool of infected cells (23). Because the expression of these cytokines is a natural occurrence in the microenvironment of lymphoid tissue, we speculated that induction of HIV by this combination of cytokines may in part explain the commonly observed reappearance of detectable plasma viremia in HIV-infected individuals in whom HAART had been discontinued (23).

Persistence of the Latent HIV Reservoir

Although there is no question that the latent HIV reservoir in the pool of resting $CD4^+$ T cells persists in HIV-infected individuals receiving HAART for considerable periods of time (11–13, 24), the precise duration of the half-life of this viral reservoir has been debated, as has the question of whether there is ongoing viral replication in those individuals in whom plasma viremia has been successfully suppressed (11, 25–28). The current estimated half-lives of replication-competent HIV in the pool of latently infected, resting $CD4^+$ T cells encompass a broad range. A recent longitudinal study has indicated that the half-life of replication-competent HIV in the pool of latently infected, resting $CD4^+$ T cells is $\approx$44 months (24). According to this estimate, eradication of HIV could take as long as 60 years of treatment with HAART, assuming that the size of this viral reservoir is only $\approx 10^5$ cells per infected person and that no other viral reservoirs exist (24). This estimate is in contradistinction to yet another estimated half-life of replication-competent HIV in the latently infected pool of resting $CD4^+$ T cells of $\approx$6.2 months, which would predict a time to eradication of virus of $\approx$7–10 years (25). Despite this wide disparity of estimates, both studies clearly indicate that the half-life of latent HIV in resting $CD4^+$ T cells far exceeds that of previous estimates (10) and that natural turn-over of this latent reservoir may not be sufficient to achieve eradication of HIV in infected individuals receiving HAART alone. In addition, there is evidence for genetic sequence evolution within the envelope region over time in some patients whose plasma viremia remains below detectable levels while receiving HAART, which strongly suggests ongoing viral replication (25). Evidence for ongoing viral replication had been previously suggested by Chun *et al.* in a study that demonstrated a substantial frequency of resting $CD4^+$ T cells carrying unintegrated HIV provirus in HIV-infected individuals in whom plasma viremia was suppressed to below the level of detectability by HAART (11). This observation was followed by a number of studies in which the presence of cell-associated HIV RNA—viral RNA associated with a cell, indicating that a virus is either attached to the cell or that the cell is infected and that the provirus in this cell is transcriptionally active—was demonstrated by reverse transcription–PCR in infected individuals receiving HAART with successful suppression of plasma viremia (26–28). It is unclear whether the low degree of viral replication was actually occurring in latently infected, resting $CD4^+$ T cells because none of these latter studies were performed on highly purified resting $CD4^+$ T cells. However, it is likely that ongoing viral replication could continue in sequestered anatomic sites inaccessible to HAART or that HAART is not completely effective in blocking all HIV replication. Under these circumstances, virus produced by infected cells might be responsible for the presence of unspliced HIV RNA in the peripheral blood mononuclear cell compartment of infected individuals receiving HAART (28). Furthermore, the presence of cell-associated HIV RNA is not surprising because no antiretroviral drugs that are currently available can inhibit transcription of HIV RNA from the integrated HIV proviral DNA in infected cells. Thus, it is possible that latently infected, resting $CD4^+$ T cells carrying integrated HIV DNA may undergo low levels of transcriptional activity of HIV without allowing complete assembly of infectious virions in the presence of a protease inhibitor. It should be pointed out that the presence of cell-associated HIV RNA does not necessarily mean that this RNA can give rise to infectious HIV (26–28).

Strategies for the Elimination of Latently Infected, Resting $CD4^+$ T Cells

There has been considerable interest in potential approaches toward diminishing, containing, or eliminating the latent reservoir of HIV in infected individuals (14–16, 29). Among such approaches is the preservation of normal HIV-specific immune function by early treatment of HIV infection before the development of significant virus-related immune dysfunction. Because a considerable proportion of patients begin treatment after substantial damage to the immune system has already occurred, this approach may not be feasible in most cases. Therefore, consideration has been given to the use of immune enhancers to partially reconstitute immune competency. Considering the metabolically inactive nature of the latent HIV reservoir (18, 19, 30), many of the proposed strategies directed toward elimination of latently infected, resting $CD4^+$ T cells involve direct or indirect activation of this pool of infected cells (14–16, 29). All of these proposed strategies are based on the assumptions that the half-life of productively infected $CD4^+$ T cells is relatively short *in vivo* (10, 31–33) and that infectious virions that are released during the process of activation of the latent reservoir can be efficiently contained by HAART. To achieve this goal, the use of immune-activating reagents, such as anti-CD3 antibody, IL-2, and therapeutic HIV-specific vaccines has been considered. Initial attempts at utilizing anti-CD3 antibody in HIV-infected individuals who are receiving HAART have been somewhat discouraging because of the toxic nature of this reagent *in vivo*.† The use of intermittent IL-2 in infected individuals has been extensively studied and has resulted in considerable expansion of the pool of $CD4^+$ T cells, despite manageable toxicities during the period of administration (34–42). In this regard, we had previously demonstrated that IL-2 as part of a combination of cytokines including TNF-α and IL-6 could induce the activation of virus from latently infected, resting $CD4^+$ T cells *in vitro* (23); in addition, *in vivo* administration of IL-2 resulted in increased plasma levels of TNF-α and IL-6 in HIV-infected individuals (34). We, therefore, sought to determine the effect of *in vivo* administered IL-2 on the pool of latently infected, resting $CD4^+$ T cells and recently demonstrated, in a nonrandomized study, that there is a marked diminution of this pool of cells in patients receiving HAART together with intermittent IL-2 compared with those who received HAART alone (43). To accurately determine the frequency of resting $CD4^+$ T cells carrying replication-competent HIV in infected individuals in this study, we utilized a high input coculture technique in which replicates of 10 million resting $CD4^+$ T cells were subject to *in vitro* activation followed by assay for p24 in the culture supernatant. Among those patients who were receiving IL-2 plus HAART, we identified three patients in whom replication-competent HIV was completely undetectable despite the fact that unusually large numbers (up to 360 million) of resting

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$CD4^+$ T cells were subjected to the high input coculture assays. The inability to demonstrate replication-competent HIV in the peripheral blood pool of resting $CD4^+$ T cells in these individuals was underscored by the failure to demonstrate replication-competent virus in the lymph nodes of two of these patients. Although we had demonstrated that intermittent IL-2 administration together with continuous HAART had resulted in a diminution of the pool of latently infected, resting $CD4^+$ T cells to the point where we could not detect replication-competent virus in these cells by available culture techniques, the question still remained concerning whether HIV had been completely eradicated in these individuals.

Failure to Eradicate HIV

There have been anecdotal reports of viral rebound after interruption of HAART in infected individuals in whom profound suppression of plasma viremia was achieved, suggesting that absence of detectable plasma viremia does not necessarily reflect eradication of HIV (44). Indeed, the proof of functional eradication of cellular reservoirs of HIV will only come from clinical trials in which therapy is discontinued in individuals who have become aviremic on therapy. Harrigan *et al.* recently reported results of such a trial and have shown that all six infected individuals who were receiving HAART for considerable periods of time and in whom the drugs were discontinued experienced a rapid viral rebound (45). Thus, it is becoming clear that eradication of HIV even in individuals whose plasma viremia had been suppressed to below detectable levels for prolonged periods of time by HAART will be extremely problematic.

Additional Potential Reservoirs of HIV

Since we and others have demonstrated that the pool of latently infected, resting $CD4^+$ T cells persists for prolonged, and perhaps indefinite, periods of time in infected individuals whose plasma viremia is successfully suppressed by HAART (11–13, 24, 25), this viral reservoir is considered to be a major impediment to the long-term control of HIV infection. Furthermore, other potential tissue reservoirs of HIV exist and include the brain, gut-associated lymphoid tissue, bone marrow, and genital tract, among other organs (29, 46). In addition, although it is clear that a pool of latently infected, resting $CD4^+$ T cells exists *in vivo*, the pathogenic significance of this latent HIV reservoir as well as its role in the rebound of plasma viremia after discontinuation of HAART remains to be fully delineated. Studies designed to determine the source of the rebound in plasma HIV after discontinuation of HAART in infected individuals will require extensive genotypic and phenotypic virologic analyses.

The Task Ahead

Although eradication of HIV in infected individuals appears to be extremely problematic at present, the goal of long-term containment of viral replication and, hence, the prevention of further virus-induced immune dysfunction should not be abandoned. It is clear that treatment of HIV-infected individuals for prolonged periods of time, and perhaps indefinitely, with currently available regimens of HAART will be extremely difficult because of the potential and reality of cumulative toxicities (47) as well as the enduring possibilities of the emergence of microbial resistance to the drugs (47) in a setting in which a low-level of persistent virus replication is highly likely (11, 25–28). In addition to more effective and less toxic HAART regimens, including, perhaps, regimens of structured drug holidays, such a goal will almost certainly require a major contribution from host factors such as HIV-specific immune responses. A number of studies have been aimed at enhancing both HIV-specific and nonspecific immune responses by a variety of approaches, including intermittent infusions of immuno-enhancing cytokines such as IL-2 (34–42), thymic transplantation (48), bone marrow transplantation (49), and, postinfection, HIV-specific vaccination (50). Many of these approaches had been attempted during the pre-HAART era, when adequate control of HIV replication was not attainable. Now that prolonged suppression of HIV plasma is plausible, such adjunctive immune-based approaches might have a much higher probability of success. In this regard, the immune system may ultimately be capable of controlling the spread of virus from HIV reservoirs, as suggested in studies of HIV-infected individuals with long-term nonprogressive disease in whom persistent HIV-1 specific $CD4^+$ T cell responses have been demonstrated (51). In fact, although it was originally felt that the immune system could not undergo a substantial degree of spontaneous reconstitution during HIV infection (52–54), it is now clear that, in the era of HAART, varying degrees of improvement in immune system function are indeed possible (55–59). Of note, the regeneration of non-HIV-specific responses appears to occur much more readily than the regeneration of HIV-specific responses (60, 61). Clearly, heightened emphasis on research on immune-based therapy directed toward the preservation and enhancement of HIV-specific immunity is warranted if long-term control and perhaps functional eradication of HIV are to be realized. Unfortunately, currently available regimens of HAART alone do not seem capable of achieving such a goal.

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