New Developments in the Pathogenesis of HIV-1 Infection: Viral Dynamics and a Reservoir for HIV-1

Characteristics of latent HIV-1 infection in resting CD4+ cells and implications for antiretroviral treatment were discussed by Robert F. Siliciano, MD, PhD. The following article summarizes his presentation.

With the advent of potent antiretroviral therapy and the ability to reduce plasma viral load levels below the limits of detection of available sensitive assays for prolonged periods, it was initially hoped that prolonged treatment could eradicate HIV-1 from the body. Initial studies showing that rapid and dramatic decreases in plasma viral load could be achieved with single potent antiretroviral drugs provided important information on viral and cellular dynamics of infection. These studies indicated that HIV-1 infection is a dynamic process consisting of new rounds of infection and replication in susceptible cells with rapid decay of both free virus and productively infected cells. The rapid decline in viral load suggested that new infection of cells was prevented, revealing the intrinsic decay rate of the various cellular compartments of the virus. Analysis of decay rates in these studies and in subsequent studies using potent antiretroviral therapy showed that an initial rapid decay phase was followed by a slower decay phase, with the second phase being attributed to turnover of chronically infected cells (Figure 1). With the ability of potent antiretroviral therapy to maintain plasma viral load below limits of detection of available assays, it was hypothesized that given continuation of this second decay rate, virus could potentially be completely eliminated with 2 to 3 years of therapy.

However, the nature of viral dynamics when plasma viral load is below assay detection limits remained undefined. Evidence of ongoing viral replication in patients on potent antiretroviral therapy comes from the following observations: (1) not all patients on therapy achieve plasma viral loads below limits of detection and (2) virologic failure can occur in patients fully suppressed on therapy upon switching treatment to a less intense regimen. Such findings suggest that although current potent antiretroviral regimens can reduce plasma viral load below detection limits, ongoing viral replication persists. One form of viral persistence consistent with these observations would be a low-level 'smoldering' infection in CD4+ cells. Other mechanisms that might account for viral persistence consist of reservoirs of persistent HIV-1. Identified reservoirs include follicular dendritic cells, located in the germinal centers of peripheral lymphoid tissue. These cells can retain antigenic material on their surface for prolonged periods, with half-life of this reservoir having been estimated at approximately 2 weeks. Persistently infected macrophages constitute a second potential reservoir; since HIV-1 infection does not kill these cells, they may continue to produce virus for their life span. The half-life of macrophages is approximately 2 weeks, and mathematical models have suggested that the second phase of decay in plasma viral load under antiretroviral treatment is due to turnover of these cells. The most significant of viral reservoirs appears to be resting memory CD4+ cells bearing an integrated copy of the viral genome. Since these cells have an exceptionally long life span, this reservoir would appear to represent a major barrier to the goal of viral eradication.

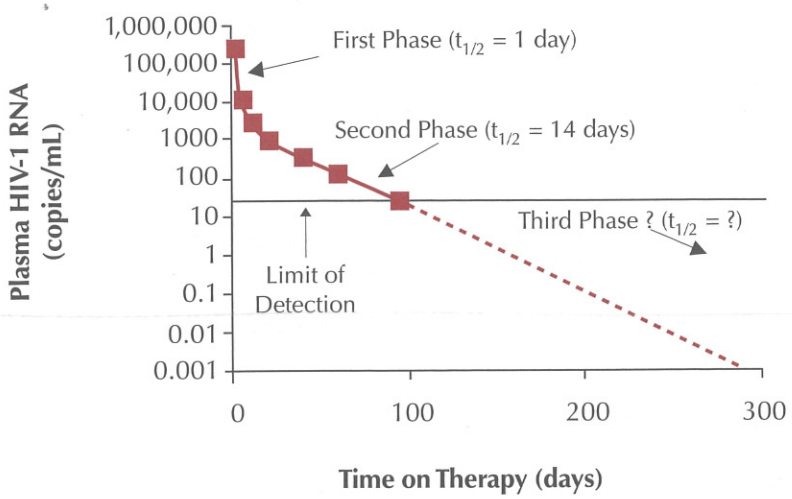

Figure 1. *Representation of 2 identified phases of plasma HIV-1 RNA decay with introduction of potent antiretroviral therapy and reduction of viral load to levels below detection limits of the assay. The first phase is attributed largely to turnover of infected activated CD4+ cells and the second largely to turnover of infected macrophages or other chronically infected cells. Adapted with permission from Finzi D, et al.* Cell. *1998;93(5):665–671. Copyright 1998 Cell Press.*

ESTABLISHMENT OF LATENT INFECTION IN CD4+ CELLS

CD4+ T cells emerge from the thymus and enter the circulation as naive cells. Upon encountering antigen, they are activated and proliferate, carrying out various immunologic functions in response to the initiating antigen (Figure 2). After several rounds of division, some of these cells revert to an inactive state as memory T cells that permit an immune response in subsequent cases of exposure to the activating antigen. Each of these stages of T-cell development is permissive for HIV-1 infection. Whereas infection of activated CD4+ cells results in productive infection, latent infection may be established in resting cells via 2 mechanisms (Figure 3). Preintegration latency is a transient form of latency in which nuclear import of transcribed viral DNA does not occur after fusion of the virion with a resting CD4+ cell and reverse transcription of viral RNA. If the cell becomes activated before HIV-1 genomes in the cytoplasm are degraded (a process occurring over hours to days), nuclear import, integration into chromosomes, virus gene expression, and assembly and release of infectious virus can occur. The more stable postintegration latency can occur when activated CD4+ cells, in which integration of proviral DNA has occurred, revert to a resting state with minimal transcription of viral genes; such cells must survive both the cytopathic effects of the infection and cytolytic host effector mechanisms. As discussed below, studies have now definitively established the existence of a pool of such cells in infected individuals. In theory, activation of these cells would result in productive infection at low levels; although virus produced from such a reservoir would constitute only a small proportion of plasma virus in untreated individuals, persistence of the virus in this reservoir assumes considerable importance in patients in whom viral load is profoundly suppressed with potent antiretroviral therapy.

Postintegration latency can occur when activated CD4+ cells, in which integration of proviral DNA has occurred, revert to a resting state with minimal transcription of viral genes

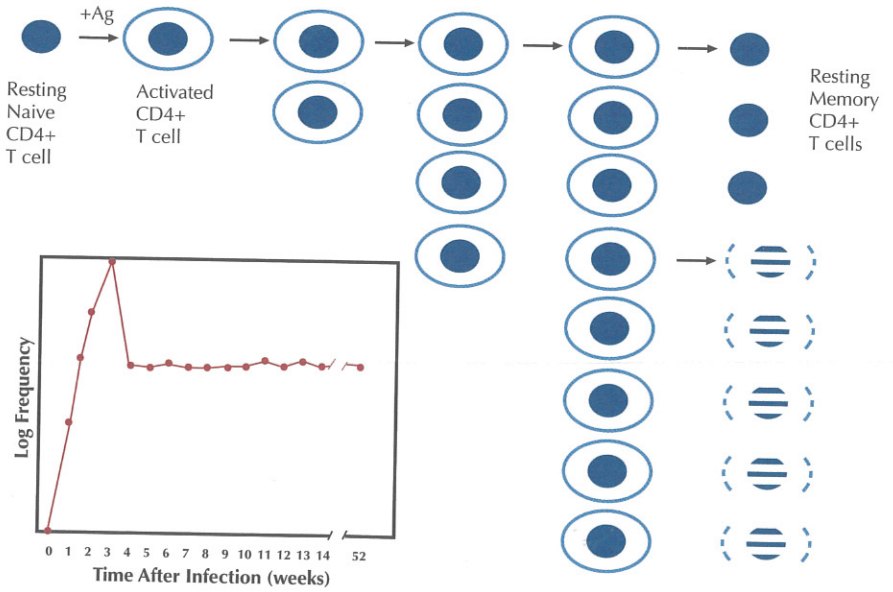

Figure 2. *Representation of activation, proliferation, and deletion or reversion to inactive (memory) state of CD4+ T cells in response to infection. Inset shows frequency of antigen-specific cells during and after immune response to infection.*

LATENT RESERVOIRS IN BLOOD AND LYMPHOID TISSUE

Studies to detect resting CD4+ T cells with integrated viral DNA were conducted by isolating high-purity populations of resting cells and utilizing an inverse polymerase chain reaction to amplify a segment of the proviral DNA. Studies in peripheral blood from HIV-1-infected donors showed that cells with integrated viral DNA constituted less than 0.01% of resting CD4+ cells. Additional studies of samples of peripheral blood and lymph nodes from patients with asymptomatic HIV-1 infection showed that the frequencies of such cells were similar in blood and lymph node tissue, ranging from less than 16 to 410 cells per million resting cells in the latter, and were not correlated with CD4+ count, plasma HIV-1 RNA level, or antiretroviral therapy. Subsequent studies, utilizing a novel quantitative culture assay to determine what proportion of these cells could produce infectious virus, showed mean frequencies of replication-competent virus of 5 and 7 per million resting cells in lymph node and blood, respectively, with these reduced estimates suggesting the presence of defective viral DNA in some cells (Figure 4). Estimates of the total body number of resting cells with integrated viral DNA based on these data indicate a range of from 4.6×10^6 to 3.4×10^7, with a mean of 1.2×10^7.

PERSISTENCE OF LATENT INFECTION IN PATIENTS ON POTENT ANTIRETROVIRAL THERAPY

Although the size of the pool of resting CD4+ cells with replication-competent virus is small, the long life of these cells

suggests that the reservoir represents an enduring potential for rekindling of infection. In a recent study, Dr Siliciano's group evaluated the presence of latent virus in a selected group of 22 patients who were highly adherent to their antiretroviral therapy regimen. These patients had received a 3- to 5-drug regimen including a protease inhibitor for up to 30 months, had exhibited increased CD4+ cell count and a rapid decline in plasma HIV-1 RNA to levels below detection (<200 copies/mL) that was consistently maintained over the course of treatment, and were heterogeneous with respect to age, sex, risk factor for infection, disease stage, prior treatment, and current antiretroviral regimen. Dr Siliciano's group used a culture method in which resting CD4+ T cells from these patients were exposed to optimal conditions for activation prior to co-culture. This method showed that replication-competent virus was present in all 18 cases in which a sufficient number of resting cells were available for evaluation, with frequencies ranging from 0.2 to 16.2 cells per million. Cross-sectional analysis (Figure 5) showed that the frequency of resting cells with replication-competent virus did not decrease according to time on therapy, suggesting relative stability of this reservoir with potent antiretroviral therapy.

In a subsequent study, Dr Siliciano's group attempted to directly measure the decay rate of latently infected cells by following patients longitudinally. In a group of 42 patients receiving potent antiretroviral regimens, latently infected resting cells were detected in 33 of 35 who had responded to therapy with a decrease in plasma HIV-1 RNA level to less than 200 copies/mL and maintenance of viral load below this detection limit. After an initial decline in frequency of latently infected cells due to turnover of those cells with preintegration latency during the initial weeks of treatment, frequencies clustered around a value of 1 cell per million (Figure 6). These remaining cells appear to decay quite slowly, with longitudinal follow-up indicating a half-life value on the order of 44 months. At this decay rate, eradication of this reservoir would require 60 years based on estimates of the total body number of resting cells with latent replication-competent virus. Of note, this estimated half-life has been repeatedly revised upward with continued follow-up as subsequent measurements in patients with an apparent more rapid decay have indicated return of the frequency value to that of previous measurements. This finding is likely attributable to the fact that measured values are generally close to the assay detection limits and that there is a wide 95% confidence interval surrounding each individual measurement. Thus it is likely that little or no change in actual number of latently infected cells has occurred. In addition, low levels of ongoing replication (occurring below the level of detection of the assays) could contribute to the generation of new latently infected cells with prolongation of the apparent half-life of this reservoir. It should also be noted that another group has reported a more rapid decay in some patients, with a half-life of approximately 6 months being reported in a small subset of patients who were treated within 90 days of infection.

The pool of cells with postintegration replication-competent virus appears to decay quite slowly, with longitudinal follow-up indicating a half-life value of approximately 44 months

To determine whether virus from this reservoir had developed resistance to combination treatment, nucleotide sequences of the HIV-1 *pol* gene were assessed in isolates from a subgroup of

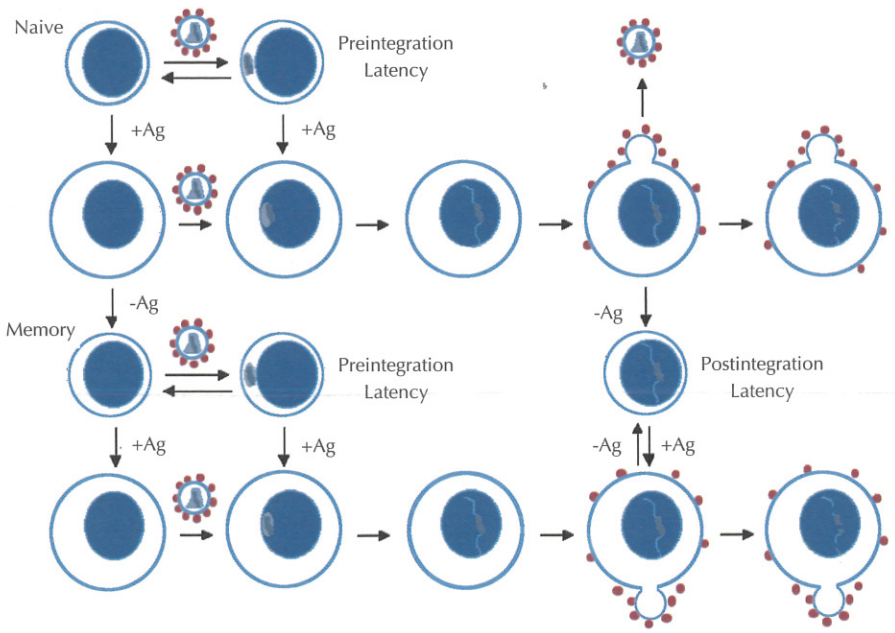

Figure 3. *Schematic of preintegration latency and postintegration latency of HIV-1 in resting memory cells. Activation by reexposure to antigen may result in productive infection in both cases. Adapted with permission from Finzi D, et al.* Cell. *1998; 93(5):665–671. Copyright 1998 Cell Press.*

patients, with results indicating that the virus from these cells were of drug-susceptible genotypes. These findings support the notions that potent antiretroviral therapy was successful in suppressing viral replication and selection of resistant mutants and that the isolates are derived from long-lived cells infected prior to initiation of potent therapy—notions consistent with the hypothesis that these cells serve as a stable, enduring reservoir for HIV-1. This scenario is also supported by observations of a patient in whom viral load under therapy with ritonavir/ saquinavir/zidovudine/lamivudine after initial failure of ritonavir monotherapy has occasionally been above detection limits. To test the hypothesis that the reservoir of latently infected cells might be reseeded by ongoing active infection, genotypic analysis of isolates was performed to detect the presence of resistant variants that might be expected to accumulate in these cells in the setting of ongoing replication. It was found that each set of isolates from this patient contained predominantly wild-type sequences with some variants that may have emerged under initial ritonavir therapy, although the frequency of the latter has remained unchanged over time. Such findings suggest that this reservoir is established during primary infection with largely wild-type virus and is relatively stable thereafter. The idea that early treatment might prevent establishment of this reservoir is not supported by findings to date; several of the patients assessed in these studies were treated during primary infection, including a patient in whom treatment was started within 48 hours of presentation with acute retroviral syndrome, and latently infected cells have been identified in all of these patients.

Evidence suggests that the latent reservoir is established during primary infection with largely wild-type virus and is relatively stable thereafter

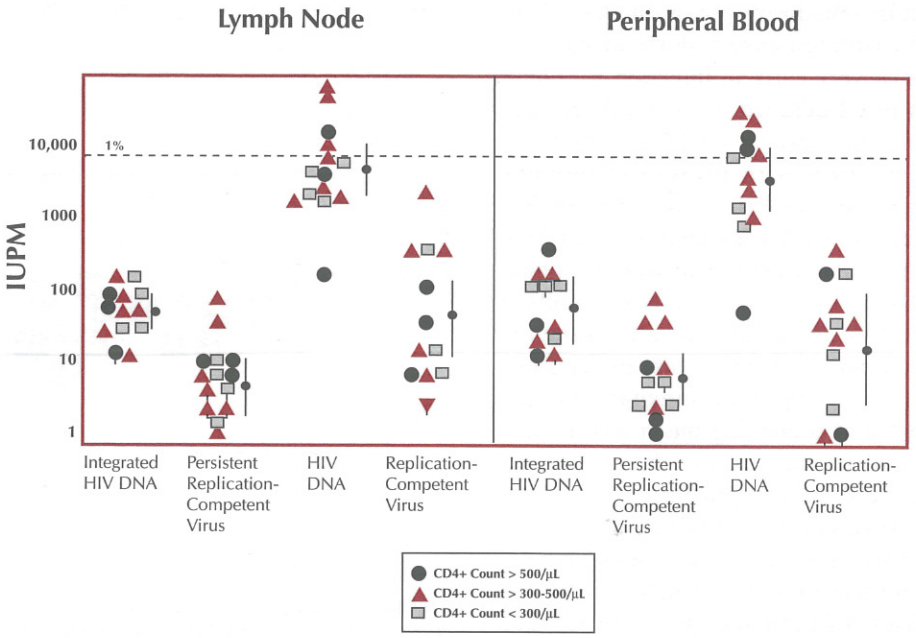

Figure 4. *Frequency of integrated HIV-1 DNA and replication-competent virus in resting CD4+ T cells in lymph node tissue and peripheral blood. IUPM indicates infectious units per million cells. Adapted with permission from Chun T-W, et al.* Nature. *1997;387:183–188.*

PROSPECTS FOR ERADICATION OF THE LATENT RESERVOIR

A recent report by investigators from the National Institutes of Health suggests that eradication of the latently infected CD4+ cells may be possible with the addition of interleukin-2 (IL-2) to antiretroviral therapy to activate the resting cells, resulting in cell death from viral cytopathic effect or from immune mechanisms. In this report, latently infected cells became undetectable in 3 of 14 patients treated in this manner. In addition to the possibility that latently infected cells persisted in these patients at a frequency below assay detection limits, there are a number of issues regarding the potential for success of strategies involving IL-2 use. One is that resting cells (ie, CD25-phenotype) do not express high-affinity IL-2 receptors (but do express low-affinity receptors), leaving it generally unclear how the effects of IL-2 in activating the cells might be mediated. Second, it is unclear what fraction of these cells might become activated by IL-2, an important consideration given that all of the cells must become activated for eradication to be achieved. Finally, the fate of the cells that become competent for transcription of the latent provirus is also unclear, with the mechanism of clearance in vivo remaining undefined. Continued investigation of the effects of IL-2 or other methods of activating latently infected cells in this setting will provide a better idea of the potential utility of such an approach.

It is also possible that the potential adverse consequences of the existence of this reservoir might be avoided through methods to enhance effector immune response. Studies in long-term nonprogressors with HIV-1 infection indicate the presence of a strong HIV-1-specific

cellular immune response, and there have been isolated case reports of patients treated very early in infection who have exhibited delayed or no viral rebound upon withdrawal of treatment in apparent association with preserved immune response. Dr Siliciano's group has detected latently infected resting CD4+ cells in one such patient who has maintained a plasma viral load below limits of detection for 33 months after stopping treatment, suggesting that under some conditions immune mechanisms are capable of preventing this reservoir from rekindling infection in the absence of continued antiretroviral therapy.

It should be noted that attempts to identify latently infected cells have not been successful in all patients, including subsets of patients who were rescued from advanced disease by potent antiretroviral therapy, who had a low number of such cells prior to therapy, or who were long-term nonprogressors, in addition to those patients receiving antiretrovirals plus IL-2 mentioned above. Although it is probably the case that in each of these instances levels of latently infected cells simply are below limits of detection, it has been the experience of Dr Siliciano's group that it is often difficult to identify such cells in patients who initiated effective antiretroviral therapy in very advanced disease—eg, at CD4+ cell counts below 10/μL—and responded with CD4+ cell count increases of large magnitude. It is possible that immune reconstitution in such patients includes new cells that do not acquire latent infection, raising the hope that generation of latent infection may not be a general consequence of immune reconstitution.

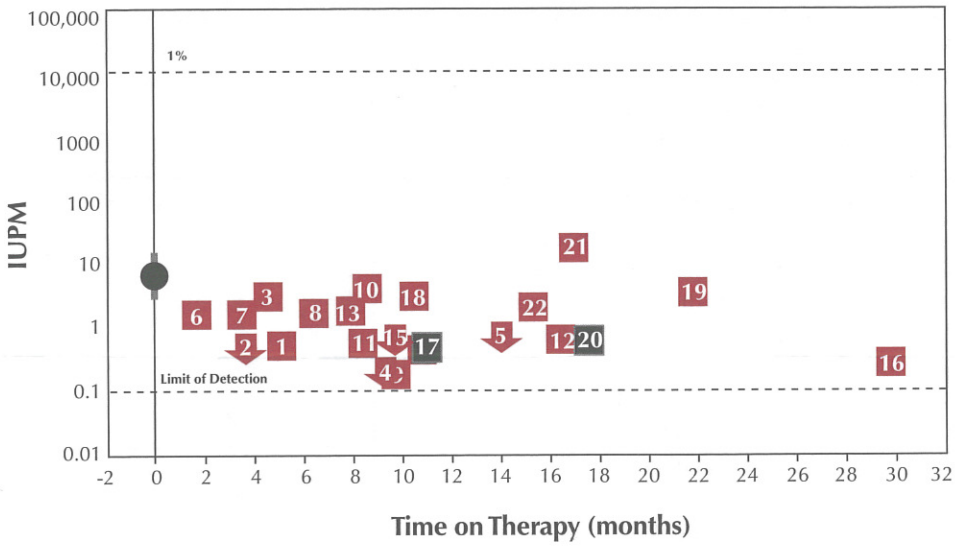

Figure 5. *Cross-sectional analysis of frequency of latently infected resting CD4+ T cells in individual patients by time on potent antiretroviral therapy. IUPM indicates infectious units per million cells. Adapted with permission from Finzi D, Hermankova M, Pierson T, et al.* Science. *1997;278:1295–1300. Copyright 1997 American Association for the Advancement of Science.*

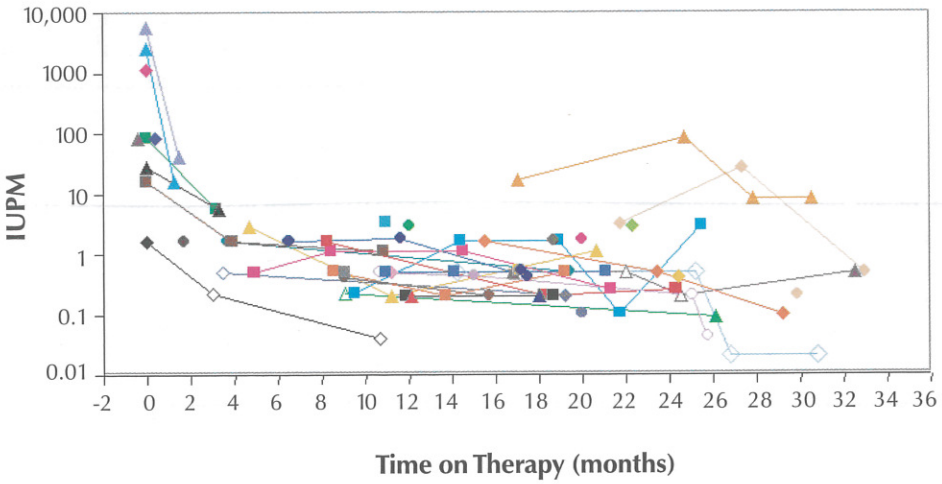

Figure 6. *Longitudinal analysis of frequency of latently infected resting CD4+ T cells by time on potent antiretroviral therapy. IUPM indicates infectious units per million cells. Adapted from Finzi D, et al.* Nat Med. *1999;5(5):512–517.*

CONCLUSIONS

The findings discussed indicate that HIV-1 can establish a state of latent infection in long-lived resting memory CD4+ T cells, with this reservoir persisting in patients taking potent antiretroviral regimens who have maintained plasma viral load below limits of detection for prolonged periods. This reservoir appears to be established early in infection and to be relatively stable over time; the current estimate of the half-life of this reservoir is 44 months, indicating the potential survival of these cells for the lifetime of infected individuals. Although it is known that memory T cells are long-lived, the life span of such cells in uninfected individuals has not been established. It is known that functional memory for viral infections can persist for at least 60 years, although it is unknown whether this long-term memory reflects survival of memory cells. Studies of memory T cells with dicentric chromosomal lesions, which die during mitosis, have indicated an intermitotic half-life of approximately 5 months. However, given the significant disruption of T-cell homeostasis that occurs in HIV-1 infection, it is unclear whether these findings can be applied to

infected individuals. Additional studies to define more clearly the decay rate of this reservoir are needed.

The implications of the long-term persistence of latently infected cells include the possibility of a rekindling of infection if antiretroviral therapy is stopped. On the other hand, the early findings indicating that the virus in this reservoir in patients on potent antiretroviral therapy represents drug-susceptible virus indicates that therapy is capable of completely suppressing active viral replication and emergence of resistant variants; thus, continued suppression of virus from the latent reservoir should be possible with continued treatment. Additional investigation of methods for eradication of virus from latent reservoirs is needed.

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Suggested Reading

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