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HIV in the Lymph Tissue and Latent Long-lasting Virus in T Cells (CD4s) **by Dr. Robert Siliciano, MD PhD Johns Hopkins University School of Medicine**

In 1995, George Shaw at the University of Alabama and David Ho at the Aaron Diamond AIDS Research Center in New York published profoundly influential papers in the British journal *Nature* describing the effects of potent new inhibitors of HIV-1 reverse transcriptase and protease on plasma viremia in HIV-1-infected patients^{1,2}. These papers not only changed the course of AIDS therapy but also provided critical insights into the pathogenesis of the disease. The studies demonstrated that these drugs produce a very rapid and dramatic drop in the level of plasma virus. Typically, plasma virus levels decreased 100 fold in two weeks. Analysis of this rapid decline led to several important conclusions. First, HIV infection is a dynamic process characterized by new rounds of viral infection and replication in susceptible cells with rapid decay of both free virus and of the vast majority of productively infected cells. Second, the rapid decline in plasma virus suggested that the drugs were actually preventing new infection of susceptible cells, revealing the intrinsic decay rates of the various intracellular and extracellular compartments in which the virus is harbored. Although resistance developed rapidly in patients treated with these agents as monotherapy, further studies showed that combination therapy could produce a decline in plasma virus to undetectable levels in many patients. Analysis of the decay rates suggested that after the rapid initial decline in plasma virus, there was a second slower phase of decay which was attributed to the turnover of chronically or latently infected cells. Based on the observed rates of decay, David Ho, Martin Markowitz and Alan Perelson suggested that complete eradication of the infection might be possible with two to three years of highly active antiretroviral therapy (HAART)³. However, these investigators also noted, as a caveat to these optimistic predictions, that additional reservoirs for HIV not detected by standard methods might make eradication more difficult.

The work of Ho and colleagues highlighted the importance of long-lived reservoirs for HIV. There are at least three potential reservoirs. Extracellular virus particles can be trapped on specialized cells in the germinal centers of the peripheral lymphoid tissues. These cells, known as follicular dendritic cells (FDC), are able to retain antigenic material on their surfaces for long periods of time. While there is evidence that trapped virions bound to FDC can retain infectivity, this reservoir declines fairly rapidly with a half-life of about 2 weeks⁴. Persistently infected macrophages represent a second potential reservoir for the virus. Because HIV does not kill infected macrophages, these cells can in principle continue to release virus for their normal life span. In uninfected individuals, the half-life of macrophages is estimated to be about 15 days. Mathematical models constructed by Perelson and colleagues suggest that in treated individuals, the second phase of decline in plasma virus is due the turnover of infected macrophages, with an estimated $t_{1/2}$ of 14 days³.

The third and potentially most significant reservoir for HIV consists of resting memory CD4⁺ T cells carrying an integrated copy of the viral genome^{5,6}. The importance of this reservoir derives from a fundamental aspect of the biology of these cells. As is discussed below, these T cells must survive for long periods of time in order to provide the host with immunologic memory, the capacity to respond rapidly to previously encountered infections. Although reactivation of virus from this latent reservoir normally contributes only a minute fraction of the plasma virus in untreated individuals, this reservoir assumes tremendous significance in patients who are on HAART. Because of the potential of latently infected CD4⁺ T cells to survive for months to years, the reservoir may represent the major barrier to virus eradication.

Latently infected CD4⁺ T cells

The existence of a latent reservoir for HIV in CD4⁺ T cells can be best explained by considering the infection of CD4⁺ T cells in the context of the normal physiology of T cell activation. Naive CD4⁺ T cells exit the thymus and enter the peripheral lymphoid tissues where they persist in a resting state until they

encounter a foreign microorganism or antigen they are programmed to recognize. Following initial exposure to antigen, naive T cells are activated and begin to proliferate and carry out various immunologic functions. After several rounds of division, some of the activated cells stop proliferating and revert to a resting state in which they persist as memory T cells capable of responding to subsequent exposures to the initiating antigen.

Interestingly, HIV can infect CD4⁺ T cells in all of these different stages, but only activated CD4⁺ cells are fully permissive for viral replication. HIV can establish a state of latent infection in resting CD4⁺ T cells. This may occur through two mechanisms. A "pre-integration" form of latency is observed following infection of resting CD4⁺ T cells. HIV virions can bind to and fuse with resting CD4⁺ T cells, with the subsequent reverse transcription of the genomic viral RNA. However, productive infection does not result because nuclear import of the reverse transcribed viral DNA does not occur⁷. Thus, in resting T cells, partially or completely reverse transcribed HIV genomes reside in the cytoplasm for a finite period of time (hours to days) before being degraded. This pre-integration form of latency may represent an inducible reservoir for HIV-1. If an infected resting T cell is activated by antigen before the unintegrated HIV DNA becomes non-functional, then the subsequent steps of nuclear import, integration into host chromosomes, virus gene expression, and release of infectious virions can all occur.

A more stable form of latency may occur in CD4⁺ T cells that have undergone integration of proviral DNA. The state of post-integration latency may be established if productively infected, activated CD4⁺ T cells return to a resting state in which there is minimal transcription of viral genes. Because integration is dependent upon T cell activation, post-integration latency can, in principle, only result from the return of an activated T cell with integrated provirus to a resting state. In order for a productively infected CD4⁺ T cell to enter a condition of post-integration latency, it must survive *both* the cytopathic effects of the virus and cytolytic host effector mechanisms for long enough to allow the cell to revert to a resting memory state with minimal transcription of HIV-1 genes. Recent studies carried out at Johns Hopkins have provided definitive evidence that resting memory CD4⁺ T cells harboring integrated provirus are present in infected individuals⁵. Although present at low frequency, a fraction of resting CD4⁺ T cells with integrated HIV-1 DNA can produce infectious virus upon stimulation. Additional studies, described below, show that these cells can persist for at least 30 months in individuals on HAART. These results have suggested that this cellular reservoir for HIV may represent the major barrier to virus eradication in patients on HAART.

Life-span of memory CD4⁺ T cells

The notion that latently infected resting memory CD4⁺ T cells carrying integrated HIV-1 DNA might represent a long term reservoir for the virus is consistent with the biology of these cells. The life span of memory T cells in uninfected individuals is not yet well established, but it is clear that immunologic memory can persist for long periods of time. Studies of isolated human populations that are re-exposed to epidemic viral illness after a long interval suggest that functional memory for a viral infection such as measles can persist for at least 60 years. However, it is unclear whether long term memory reflects the long term survival of memory cells. There is currently an active debate over whether memory results from the long term survival of memory cells in the absence of antigen stimulation, the restimulation of memory cells by cross-reacting environmental antigens, or the restimulation of memory cells by low levels of persistent antigen. Recently attempts have been made to measure directly the life span of memory T cells. One approach to this involves the analysis of cells carrying chromosomal lesions induced by therapeutic irradiation^{8,9}. Cells carrying dicentric chromosomal lesions die during mitosis, and thus, the persistence of these cells gives an idea of the intermitotic life span of the cells. Studies of the persistence of cells with these chromosomal abnormalities indicates that memory T cells turnover with a half-life of about 5 months. However, given the substantial perturbations in T cell homeostasis in HIV-1 infection, it is unclear whether studies in uninfected individuals can be directly extrapolated. Therefore, direct measurement of the decay rate of this viral reservoir are urgently needed.

Analysis of the latent reservoir in blood and lymph node

Although it has been presumed that the integration of HIV DNA into the genomes of infected CD4⁺ T lymphocytes allows viral persistence, there has been until recently little direct evidence that CD4⁺ T cells

with integrated provirus function as a latent reservoir for HIV in infected individuals. To test this hypothesis, investigators at Johns Hopkins have developed procedures for the isolation of extremely pure populations of resting CD4⁺ T cells. Novel methods have then been used to demonstrate that integrated HIV DNA was present in a fraction of these cells. Purified resting CD4⁺ T cells were isolated using fluorescence activated cell sorting. In order to determine unambiguously whether resting CD4⁺ T cells contain integrated provirus, a novel in verse PCR assay was used to amplify the genomic DNA between the 5' end of the integrated proviral DNA and an upstream restriction enzyme site. Using this assay described above, purified resting CD4⁺ T cells from HIV-infected donors for the presence of integrated HIV DNA. An initial study of 25 donors indicated that cells with integrated HIV DNA were detectable among the resting CD4⁺ T population in peripheral blood, but only at low frequency (<0.01%)⁵. Because lymph nodes may represent a major site for viral replication^{10,11}, an additional study was conducted another study in which resting CD4⁺ T cells were purified from both blood and lymph node⁶. Organized by Dr. Patricia Barditch-Crovo, the study involved 14 asymptomatic, HIV-infected donors. The results confirmed that resting CD4⁺ T cells with integrated HIV DNA were present in infected individuals, but only at low frequency. In the lymph nodes, the frequencies ranged from a maximum of 410/10⁶ resting CD4⁺ T cells to a minimum of less than 16/10⁶ cells, the lower limit of detection. Surprisingly, the frequencies were not significantly different in blood and lymph node and did not correlate with CD4 count, plasma RNA, or therapy. These findings suggest that in asymptomatic HIV infection, a relatively stable systemic steady state is established in which only a minute fraction (<0.05%) of the resting CD4⁺ T population carries integrated HIV DNA. To provide an independent confirmation for the low frequency of cells with integrated HIV DNA and to determine what fraction of resting CD4⁺ T cells can be induced to produce infectious virus, a novel quantitative viral culture assay to detect resting CD4⁺ T cells carrying replication-competent forms of the virus. The mean frequency was 5/10⁶ cells in lymph nodes and 7/10⁶ cells in blood. These values provide independent confirmation that the frequency of resting CD4⁺ T cells in the post-integration state of latency is very low. The maximum frequencies of resting CD4⁺ T cells with replication-competent integrated provirus, estimated by this culture assay, were lower than the frequencies of resting CD4⁺ T cells with integrated HIV DNA, as detected by inverse PCR. This suggests that some of the integrated HIV DNA in resting CD4⁺ T cells is defective. However, the most important finding is that some of the HIV DNA in resting cells is capable replication-competent.

Using these data, estimates of the total body number of resting CD4⁺ T cells with integrated HIV DNA were made. Estimates ranged from 4.6 x 10⁶ to 3.4 x 10⁷ cells (mean 1.2 x 10⁷). The size of the latent reservoir is several orders of magnitude lower than previous estimates based on in situ PCR measurements¹¹. Nevertheless, the critical variable is the rate of decay of this reservoir.

Persistence of latently infected CD4⁺ T cells in patients on HAART

Because of the long life span of memory T cells, resting memory CD4⁺ T cells carrying replication-competent viral genomes, although rare, may represent an important long term viral reservoir in patients on HAART. Three recent studies have examined whether replication-competent virus could persist in the resting CD4⁺ T cells of patients on HAART who had no evidence of active virus replication¹²⁻¹⁴. In one study carried out at Johns Hopkins, the presence of latent virus in resting CD4⁺ T cells was examined in 22 patients treated with HAART for up to 30 months. Patients who met each of the following three criteria were selected: first, patient- and physician-reported strict adherence to aggressive HAART regimens (typically three or four drugs including a protease inhibitor); second, a rapid decline in plasma HIV-1 RNA to undetectable levels (<200 copies/ml) following the initiation of HAART; and third, continued undetectable plasma HIV-1 RNA levels on repeated measurement throughout the course of the study. Plasma HIV-1 RNA measurements fell dramatically following the initiation of treatment, reaching undetectable levels in 2-3 months, and remained at an undetectable level upon repeated sampling including the day blood was taken for analysis of latently infected cells. Patients were tested an average of four times each during the months following initiation of HAART. A subset of three more intensively studied patients had monthly measurements of plasma HIV-1 RNA levels, which were uniformly undetectable. These results suggest that in all patients selected for study, long term suppression of viral replication to undetectable levels was achieved.

To determine whether resting CD4⁺ T cells provided a long term viral reservoir in patients experiencing

sustained suppression of plasma HIV-1 RNA by HAART, highly purified populations of resting CD4⁺ T cells were isolated from the peripheral blood of these donors as described above. After two to twelve months on HAART, standard culture assays for replication-competent virus in unfractionated peripheral blood mononuclear cells (PBMC) are typically negative³. Therefore, a special culture method in which the resting cells were subjected to optimal conditions for activation prior to co-culture was developed in an effort to isolate and quantitate infectious virus in these patients. Despite the fact that conventional virus culture assays are negative in patients on long term HAART, replication competent virus was isolated in each of 18 cases where a sufficient number of resting CD4⁺ T cells were available for analysis. One of these patients had been on HAART for 30 months with no detectable plasma virus. Two other patients had been on HAART for over 20 months. The measured frequencies ranged from 0.2 - 16.2/10⁶ resting CD4⁺ T cells. In a cross-sectional analysis, frequencies of latently infected CD4⁺ T cells did not decrease appreciably with increasing time on HAART. These results suggest a slow decay rate for this compartment, consistent with the long term survival of memory CD4⁺ T cells in uninfected individuals (intermitotic life-span = 5.5 months, ref. 9).

To determine whether the viruses that persisted in latently infected resting CD4⁺ T cells had developed resistance to the relevant antiretroviral drugs, nucleotide sequences of the HIV-1 *pol* gene were determined for 12 viral isolates. Analysis of sequence data showed little evidence for evolution of resistance. These results indicate that in the patients studied, combination therapy was effective in suppressing viral replication and the attendant selection of resistant variants. The isolation of viruses with drug-sensitive genotypes from resting CD4⁺ T cells suggests that these isolates are derived from long-lived cells that were initially infected prior to the initiation of HAART, consistent with the hypothesis that this compartment functions as a stable, long-term reservoir for the virus.

Clinical implications of the latent reservoir

The results described above indicate that HIV can establish a state of latent infection in long-lived resting memory CD4⁺ T cells. This reservoir persists in patients on HAART, even those who have been aviremic for long periods of time. Because replication-competent virus can be isolated from this compartment by appropriate *in vitro* manipulations, there remains the possibility that this compartment could serve to "rekindle" the infection in treated patients who go off therapy. In other words, the existence of a small but relatively stable compartment of latently infected cells should be considered in deciding whether treatment should be stopped in patients with no other evidence of residual virus. On the optimistic side, it is important to note that the size of this compartment is small, less than 10⁷ cells. Most importantly, the virus harbored in this compartment appears, upon initial analysis, to retain a drug sensitive genotype. This result strongly suggests that in patients who respond well to HAART and who adhere closely to the regimens, it is possible to completely shut off active viral replication and the attendant development of resistance. Thus continued suppression of viruses originating from this compartment should be possible with continued therapy. Future studies to define more clearly the rate of decay of this reservoir are urgently needed.

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Speakers at the January 17th Forum included:

[Jules Levin, Executive Director of NATAP - Welcoming Address](#)

[Dr. Robert Siliciano, MD - HIV in the Lymph Tissue and Latent Long-lasting Virus in T-Cells \(CD4s\).](#)

[Dr. Justin McArthur, MBBS, MPH - The Brain, HIV, and the Effect of New Treatments](#)

[Dr. Carl Fichtenbaum, MD - Treatment and Prophylaxis for Opportunistic Infections in the New Potent Therapy Era. Can Prophylaxis or Maintenance Therapy Be Discontinued?](#)

[Dr. Louise Markert, Thymus Transplant Research for HIV and Its Potential for Immune Reconstitution](#)