

# VIRAL AND CELLULAR DYNAMICS IN HIV-1 DISEASE

*At the Atlanta course, R. Pat Bucy, MD, PhD, discussed viral and cellular dynamics in HIV-1 disease, presenting a number of provocative concepts emerging from recently reported findings of others, as well as from the ongoing work of Dr Bucy's group.*

The natural history of HIV-1 disease is considered to be a continuum of progression comprising 3 general phases. The acute infection stage, lasting 2 to 3 months, is characterized by high viral load and an acute flulike illness in some cases. A dramatic decrease in plasma HIV-RNA level (viral load) then occurs coincident with the development of effective CD8+ T-cell response. The clinical "latent" disease stage, lasting from approximately 6 months to more than 20 years, is characterized by wide interindividual variability of viral load but relative stability within the individual, with the viral load being correlated with rate of loss of CD4+ cells. Clinically, this stage is marked by lymphadenopathy and constitutional symptoms. Late-stage disease is characterized by low and more rapidly declining CD4+ count, often accompanied by increased viral load, and onset of opportunistic infections. These phases feature different viral and cellular dynamics; recent findings have contributed to elucidation of these dynamics and have increased appreciation of their potential complexity.

## HOST MECHANISMS IN CONTROL OF VIRAL REPLICATION

Of dominant interest have been the host mechanisms of viral control following the increase in viral load in primary infection that determine the steady-state level of viral burden during the prolonged second phase of disease. The primary concept invoked to explain this control is that of antigen-specific immune response. The effector mechanism in this response is considered to be the CD8+ T-cell (cytolytic T-cell), the efficiency of which may be determined or modulated by HIV-1-antigen-specific CD4+ T-cell activity,

Although numerous in vitro studies have shown an association of cytolytic T-cell activity with viral replication, only very recently reported studies in simian immunodeficiency virus-infected animals have shown that in vivo depletion of CD8+ T cells results in increased viral load. Evidence for an antigen-driven immune response as the mechanism of viral control is also provided by data indicating a relationship between particular alleles

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*Potent antiretroviral therapy results in a decrease in CD8+ T-cell effector activity in association with decreased levels of viral antigen*

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of MHC class I antigens (which serve as antigen-presenting cell elements for CD8+ T cells) and both viral load during early infection and rate of disease progression. Finally, it has been shown that initiation of potent antiretroviral therapy results in a decrease in CD8+ T-cell effector activity in association with decreased levels of viral antigen. Similarly, if cessation of such therapy results in viral rebound, CD8+ T-cell activity subsequently increases.

Another potential host mechanism of viral control is the availability of CD4+ T cells for viral infection. In this concept, replication is controlled by the limited number of available activated CD4+ cells, with the variance in steady-state viral replication among individuals being determined by interindividual variance in availability of these cells. This concept is supported by mathematical models and by

the observation that viral load increases with immune activation by immunization or interleukin-2 (IL-2) administration. However, although this mechanism may contribute to the level of the viral load set point during relative steady state, the possibility that it is not a primary mechanism is suggested by a number of findings. One is that the approximately 5-log variability observed in viral load during this period does not appear to be accounted for by differences in availability of CD4+ T cells (which may exhibit a 3-fold interindividual variability). Another is that studies in lymph node tissue indicate that the absolute frequency of activated T cells is approximately 100- to 1000-fold higher than the frequency of HIV-1-infected T cells. This large excess of apparently available target cells may argue against availability of activated CD4+ cells as a limiting factor in viral load. However, it should be noted that the factors that determine what defines a susceptible activated CD4+ cell remains unclear; even in vitro, not all activated CD4+ cells are target cells for the virus.

## EFFECT OF POTENT ANTIRETROVIRAL THERAPY INDUCTION ON VIRAL AND CELLULAR DYNAMICS

Observation of viral and cellular dynamics after initiation of potent antiretroviral therapy has provided additional information on the interaction of viral and cellular factors. Initiation of potent antiretroviral therapy typically results in rapid decline in the plasma HIV RNA level, characterized by an exponential decay with an apparent half-life of 1 to 2 days (Figure 1); this initial rate of decline is consistent among individuals and independent of initial absolute viral load. After 1 to 2 weeks, the rate of decline slows to what has been termed the second-phase decline. The decrease in viral load reflects not only a decrease in circulating virus but also a decrease in HIV RNA-positive cells in lymphoid tissue, suggesting that the plasma HIV-1 RNA level reflects whole-body viral burden (Figure 2).

Along with the rapid reduction in viral load, there is a rapid increase in

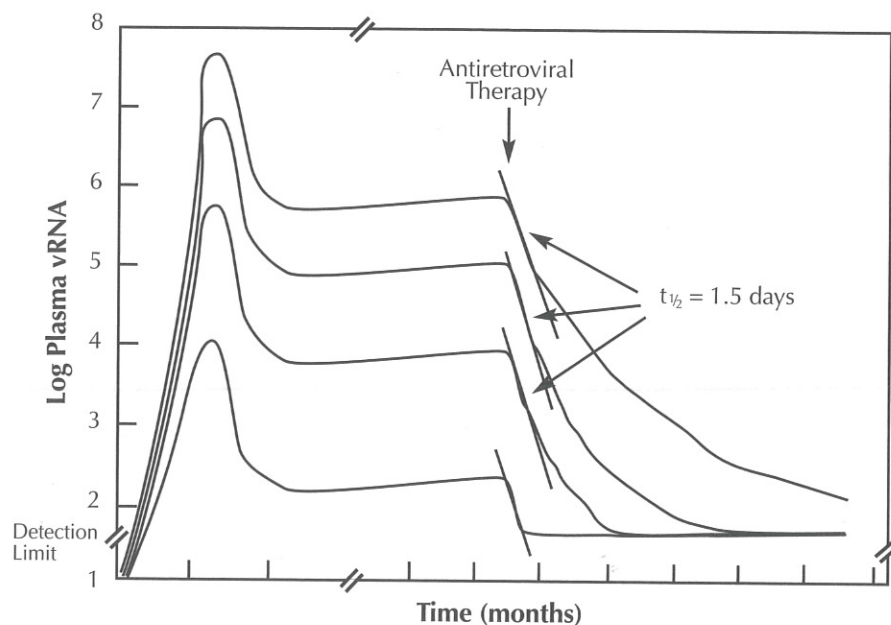


CD4+ cells in the blood. The increase appears to occur in 2 phases, a phase of rapid increase over 4 to 6 weeks and a slower phase possibly lasting for months to years. The first phase comprises a nonspecific increase in total lymphocyte count, including increases in CD4+ and CD8+ T cells and B cells, whereas the second phase may more specifically involve CD4+ T cells. Use of a new assay has shown that thymic output of new cells persists even into late life, though at attenuated levels. According to Dr Bucy, whereas there are alternative mechanisms that may explain the initial increase in total lymphocyte count, the second phase of CD4+ increase may represent a combination of expansion of existing cells and thymic generation of new cells. Particularly during the initial-phase CD4+ cell increase, there appears to be significant functional immune reconstitution, as evidenced by the acute marked reduction in constitutional symptoms. The improved immune function is subsequently associated with reductions in such clinical events as new opportunistic infections and death.

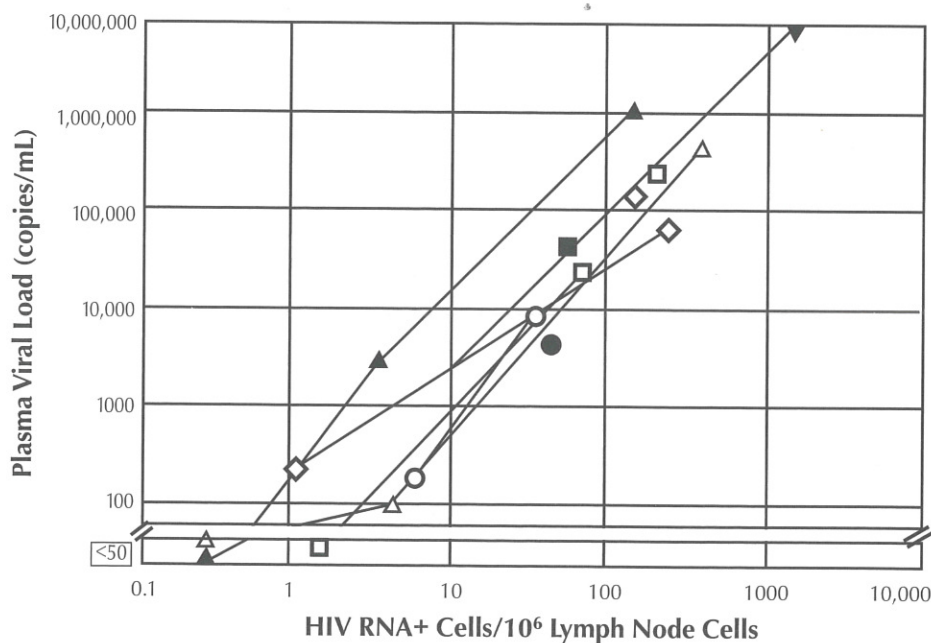
One model that was proposed to explain the CD4+ cell increase suggested that the cessation of viral replication after the initiation of potent antiretroviral therapy results in a rapid reduction in CD4+ cell death. In the context of the steady state

high viral replication and clearance in which CD4+ cell death and production are at high flux equilibrium, the increased CD4+ cell production does not cease coincident with the reduction in virus-mediated cell death, resulting in a rapid rise in the CD4+ cell population. Some of the observations of cell numbers are not consistent

with this model, like the increases in CD8+ cells and B cells observed during the phase of CD4+ cell increase. However, more recent findings, like the identification of the second-phase increase in CD4+ cell number, have resulted in a more complex picture of the CD4+ cell dynamics under antiretroviral therapy. T cells and other lymphoid cells sequestered in lymphoid tissue (eg, via increased adhesion molecule expression resulting from virus-mediated immune activation) are redistributed into the circulation upon the relief of tissue inflammation resulting from the reduction in viral antigen levels under therapy. This redistribution may account for much of the early change in CD4+ cell count. However, it is likely that increases resulting from decreased cell destruction and increased cell production, including thymic production, also begin with the suppression of viral burden upon treatment induction, with these continuing changes being identified as the second-phase increase once the early rapid rise has stabilized. The functional immune reconstitution observed may result from restoration of a more normal, less 'activated,' lymphocyte population that is consequently more functional, from the generation of new cells occurring continuously from the time at which viral load is suppressed, or from some combination of these, and perhaps other, factors.



**Figure 1.** Idealized representation of effect of potent antiretroviral therapy initiation on viral load in 4 cases characterized by relative steady-state viral loads of different magnitudes. Straight lines indicate the slope of the initial decrease in viral load after treatment is started.



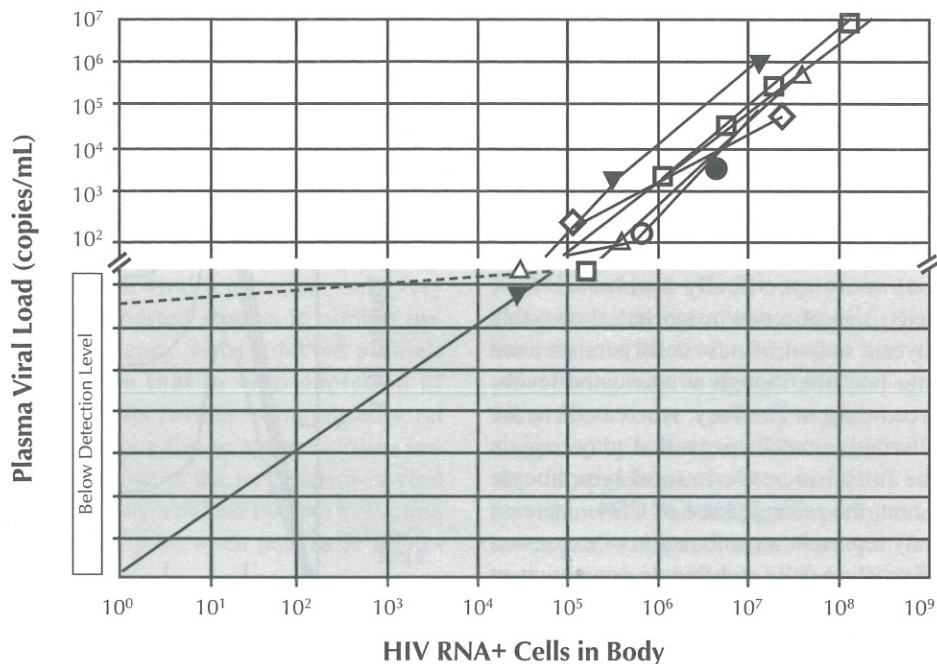
**Figure 2.** Correlation of plasma HIV-1 RNA level with RNA-positive cells in lymph nodes. Adapted with permission from Hockett RD et al. *J Exp Med.* 1999;189:1545-1554. Copyright 1999, The Rockefeller University Press.



## VIRAL DYNAMICS DURING POTENT ANTIRETROVIRAL THERAPY

Despite initial optimism, it is clear that viral infection is not eradicated with potent antiretroviral therapy, as demonstrated by the viral rebound typically observed upon withdrawal of treatment. A number of pools of residual viable virus have been identified or posited. One reservoir that has been demonstrated to exist consists of latently infected CD4+ T cells, with there also being the possibility that virus persists at sequestered anatomic sites, such as the central nervous system. It has also been suggested that virus remaining at nonsequestered sites is capable of persistent rounds of very low level de novo infection, a process that would be supported by likely intermittent nonadherence to potent antiretroviral therapy regimens. Supporting the idea of persistent viral replication are the findings of a slow evolution of viral quasi species sequences and a low rate of drug resistance mutations even under conditions of stringent adherence to treatment regimens. As pointed out by Dr Bucy, HIV-1 RNA levels below the limit of detection of 50 copies/mL for current highly sensitive assays do not ensure absence of replication: although there is a lack of consensus among investigators on this issue, one set of assumptions indicates that there could be as many as 100,000 replication-active cells producing virus at undetectable levels among an estimated whole body population of  $10^{11}$  lymph node cells (Figure 3).

*Potential viral reservoirs include latently infected CD4+ cells, sequestered anatomic sites, and a pool of virus capable of persistent rounds of low-level de novo infection*



**Figure 3.** Potential numbers of HIV-1 RNA-positive cells in the body that could still result in plasma HIV-1 RNA level below 50 copies/mL, based on correlation of plasma HIV-1 RNA level and RNA-positive cells in lymph nodes and assumption of  $10^{11}$  lymph node cells in the body. Adapted with permission from Hockett RD et al. *J Exp Med.* 1999;189:1545–1554. Copyright 1999, The Rockefeller Press.

With regard to the pool of latently infected CD4+ T cells that persists despite potent antiretroviral therapy, some groups have found that there is no apparent decrease in frequency of these cells over time in patients started on therapy during chronic infection. The half-life for turnover of these cells has been revised upward with ongoing follow-up of study patients, with current estimates of more than 20 months indicating that a period of 25 to 30 years would be required for this population of cells to be eliminated by natural turnover. One group, however, has identified a more rapid decrease in frequency of these cells, with a half-life of approximately 6 months, in patients started on potent antiretroviral therapy in early infection. In addition, another group has reported clearance of latent infection with IL-2 treatment in 2 patients followed up for a short period. In addition to the identification of latently infected CD4+ T cells, HIV-1 RNA can be detected in lymphoid cells despite its being undetectable in plasma, with viral RNA having been identified in peripheral blood monocytes, lymph nodes, gut mucosa, semen, and cerebrospinal fluid. Dr Bucy stated that it is likely that this apparent ongoing replication accounts for the rebound in viral levels upon withdrawal

of treatment. Although these viral RNA-expressing cells may arise from persistent rounds of de novo cellular infection, an alternative concept is that these rare cells arise from activated latently-infected cells that do not initiate new rounds of infection under continuing therapeutic drug concentrations.

Although most anecdotal reports of withdrawal of potent antiretroviral therapy have indicated a rapid rebound in viral load with treatment withdrawal, there have been a few highly publicized recent reports of delay or absence of rebound in patients in whom treatment was started in early infection. It is hypothesized that preservation of immune response through early potent treatment in these patients permits control of infection once treatment is stopped. One group has reported that viral rebound in patients stopping treatment was accompanied by increased cytolytic T-cell response; in 2 of 4 patients, viral load subsequently returned to and has remained at levels below detection, with 1 patient having been followed up for 23 months.

Intriguing implications are raised by the combined evidence that (1) immune response controls viral load; (2) early treatment preserves immune response (including



potentially accounting for delayed viral rebound with withdrawal of treatment); and (3) effector immune response is decreased with the reduction in viral antigen resulting from potent antiretroviral therapy. The decreased effector immune response observed with profound viral inhibition permits the persistence of low-level viral replication under potent antiretroviral therapy. One possibility in this regard is that latently infected cells that become activated are permitted to revert to a latent

phenotype owing to slowing or absence of clearance by CD8+ T cells. This scenario suggests the potential for using therapeutic immunization or similar methods to induce an active immune response as an adjunct to potent antiretroviral therapy. Most studies of therapeutic immunization were performed prior to the use of profoundly suppressive antiretroviral therapy—ie, in patients who still harbored significant amounts of viral antigen. Antigenic stimulation of the effector immune

response by a therapeutic vaccine in patients receiving potent antiretroviral therapy merits investigation to determine if it can prevent persistent replication attributable to loss of this response through absence of antigen.



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#### SUGGESTED READING

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