Perspective

Insights in HIV Pathogenesis and Antiviral Control

At the March 2002 International AIDS Society–USA course in Atlanta, H. Clifford Lane, MD, discussed aspects of the interaction between HIV and the host immune response. Greater understanding of this complex interaction has aided development of immune-based therapies and vaccines.

The immune systems of individuals with HIV infection are characterized by an immunodeficiency reflected in the loss of CD4+ T cells, coupled with immune system activation reflected in an increased T-cell turnover and functional immunosuppression. In general, the state of the immune system comprises the dynamic interactions of the activity of HIV, the HIV-specific immune response, and the regenerative activity of the immune system.

Effects of HIV and Antiretroviral Treatment on CD4+ T Cells

Characteristics of the immunodeficiency observed in HIV infection include a decline in total CD4+ T-cell number, a preferential loss of CD4+ T cells with a "naive" phenotype, and a skewing of the T-cell receptor repertoire. To understand the effects of infection on CD4+ T-cell dynamics and characteristics, it is important to know how the CD4+ T-cell pool is generated and maintained under normal conditions. Undifferentiated stem cells undergo transit through the thymus, where T-cell receptor genes in germ-line configuration are rearranged to form functional genes encoding the T-cell receptor proteins. After undergoing positive and negative selection, the cells exit the thymus capable of recognizing self and antigen, with each cell having a defined antigen specificity (conferred by expression of an immune globulin-gene-like rearranged heterodimeric receptor). The CD4+ T cells, which act to stimulate and coordinate the activity of immune effector cells, proliferate in response to processed antigen presented in association with class II major histocompatibility complex alloantigens and mitogens. The cells are considered "naive" until they encounter their specified antigens, at which point they become "memory" cells. Naive cells are characterized by surface expression of the high-molecular-weight CD45R isoform CD45RA, and memory cells are characterized by expression of the low-molecular-weight isoform CD45RO. At birth, virtually all CD4+ cells are in the naive cell pool; during subsequent increasing exposure to antigen, clonal expansion of the memory cells and constriction of the CD4+ T-cell pool results in an increasing proportion of memory cells in the total pool.

Studies in patients with HIV infection indicate that progressive reduction of the total CD4+ cell count is associated with a disproportionate reduction in the naive cell compartment (Connors et al, *Nat Med*, 1997). Under potent antiretroviral therapy, HIV-infected patients with significant numbers of both naive and memory cells exhibit immediate increases in both compartments. If there has been severe depletion of the CD4+ T-cell pool, increases are seen predominantly in the memory compartment. Such data suggest that the increase in total CD4+ cell count observed with potent therapy reflects peripheral expansion of the cells present prior to therapy.

To study characteristics of T-cell production and death, a number of investigators have used DNA-labeling techniques such as bromodeoxyuridine (BrdU) labeling. In this technique, subjects undergo a 30-minute infusion of BrdU, which is incorporated into the cellular genomic DNA, and blood or lymph tissue is then periodically sampled. Samples are stained for cell surface markers and BrdU labeling; by use of flow cytometry, the numbers of cells produced during the BrdU pulse and their decay rate following the pulse can be determined. In one study using this technique, investigators examined the ratio of lymph node to blood of labeled CD4+ and CD8+ T cells and B cells at 4 hours, 1 day, and 3 days after BrdU infusion. Their results indicate that for the population of labeled T cells, cell division immediately after labeling occurs preferentially in the lymph node. After 1 day, however, labeled cells are equally distributed between the lymph node and

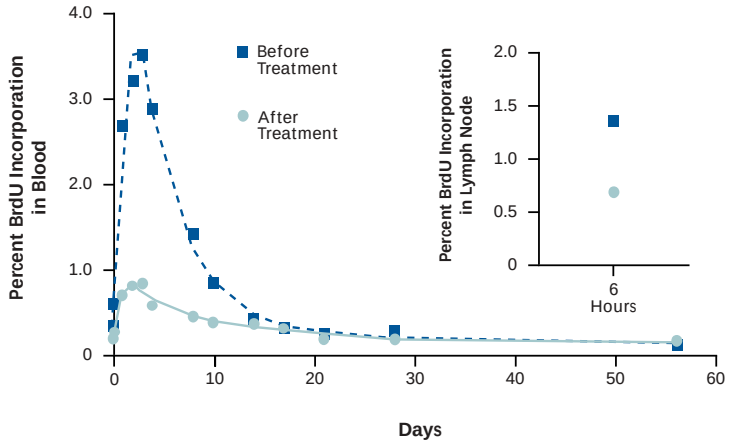

Figure **1.** Comparison of change in CD4+ T-cell production in 1 patient indicated by bromodeoxyuridine (BrdU) labeling in blood samples before and 12 weeks after initiation of potent antiretroviral therapy. Inset shows difference in lymph node sample at 6 hours after BrdU pulse. Adapted with permission from Kovacs et al, *J Exp Med,* 2001.

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the blood. These findings suggest that monitoring of T-cell characteristics in the peripheral blood provides a good reflection of characteristics in the lymphoid tissues. Extended monitoring of the labeled cells shows that the T-cell pool consists of cells with a rapid turnover rate, as well as cells with a slow decay rate.

This technique was used to assess CD4+ T-cell production before and after initiation of potent antiretroviral therapy in a group of HIV-infected patients (Figure 1). BrdU labeling before initiation of treatment and at 12 weeks after initiation of treatment showed that CD4+ cell production as measured in the peripheral blood was decreased by approximately 75% during antiretroviral therapy, with results being similar in blood and lymph node tissue. These data strongly suggest that there is no defect in CD4+ T-cell production in HIV infection. Modeling of the CD4+ and CD8+ T-cell kinetics indicates the presence of one pool of cells with rapid turnover and one with slower turnover. For the rapidly dividing pool, the size of the pool was found to be closely correlated with plasma HIV-1 RNA levels.

There is ongoing debate over whether the observed CD4+ T-cell count decline in HIV infection is attributable to decreased T-cell production or increased T-cell death. The finding that T-cell production is considerably higher and correlated with plasma HIV-1 RNA levels in untreated infection, however, indicates that the primary mechanism of depletion is increased T-cell death (whether through direct cytopathic effects of HIV or activation-induced death). The increase in the CD4+ T-cell pool observed with potent therapy appears to be due to a decrease in cell death that more than compensates for the decreased production during therapy. The conclusion that can be drawn is that HIV infection leads to a state of immune activation and increased T-cell production inadequate to compensate for cell death, and that potent antiretroviral therapy immediately reverses the virus-driven increased T-cell production and reduces the polyclonal activation associated with functional immunosuppression. This conclusion helps explain the rapid improvement in opportunistic illnesses and the immune reactivation syndromes observed with potent antiretroviral therapy.

This conclusion also provides a mechanism other than increased thymic output to account for the increases in T-cell receptor rearrangement excision circles (TRECs) with potent antiretroviral therapy. TRECs are a circularized form of DNA, created by DNA excision during the process of T-cell receptor rearrangement in stem cells, that provides an index of thymic production of T cells. Since the TREC DNA is retained in only 1 daughter cell with each cell division, each round of cell divisions dilutes concentrations of TREC-containing cells by 50%. Thus, levels of TRECs in the T-cell pool are dependent on thymic cell output and rate of cell turnover.

A number of studies assessing T-cell dynamics using TREC measurements have shown that TREC levels are decreased in patients with HIV infection compared with uninfected controls and that TREC levels increase with initiation of potent antiretroviral therapy. Such data have been interpreted to indicate that thymic output is a major source of new cells following antiretroviral therapy and that T-cell production increases with the initiation of therapy. However, when change in TREC number is assessed as a function of change in T-cell turnover using BrdU labeling, a strong correlation is observed (Figure 2). These findings suggest that the increase in TREC number observed after treatment is more likely a reflection of the decreased cell turnover (and thus decreased dilution of the TREC pool) resulting from treatment rather than the product of increased thymic output.

Evaluation of Host Immune Response

Immunologic control of viral infections is a crucial aspect of the host defense. Studies of the nature of the immune response to HIV are important in helping to better understand the pathogenesis of HIV infection and in developing immune-based therapies and vaccines. Host defense against viral infections utilizes both the innate and the adaptive elements of the immune system. As evidenced by the failure of the immune system to rid the host of HIV, the host responses are inadequate to successfully deal with this pathogen.

Understanding the host factors responsible for the control of viral infection remains an elusive goal. Various studies have examined the roles of HIV-specific CD8+ and CD4+ T cells in the response to HIV infection, but it is still the case that the best way to measure the host immune response to HIV infection is to measure the plasma level of HIV-1 RNA in the absence of treatment. This relatively simple technique affords the ability to examine the net result of all the various host factors that can control viral replication. Following primary

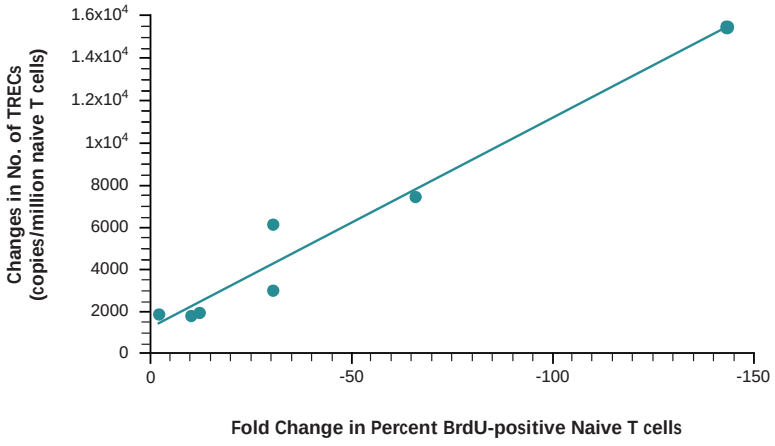

Figure **2.** Correlation between change in rates of naive T-cell turnover (fold change in percentage of bromodeoxyuridine [BrdU]-positive naive cells) and change in concentration of T-cell receptor rearrangement excision circles (TRECs) in naive T cells (R^2=0.96; *P*<.001). Adapted with permission from Lempicki et al, *Proc Natl Acad Sci USA,* 2000.

infection, a virologic set-point is reached that is difficult, if not impossible, to reduce once established. Studies in patients stopping potent antiretroviral therapy who had maintained plasma HIV-1 RNA levels at less than 50 copies/mL for periods of 1 to 4 years showed that plasma HIV-1 RNA generally returned to pretreatment levels with cessation of treatment (Figure 3). Such findings suggest that even prolonged periods of viral suppression appear to have little effect on host ability to control viral replication.

Although the determinants of the viral set-point remain unclear, 2 primary candidates are the size of the initial HIV reservoir and the number of HIV-specific T cells. A study by Davey and colleagues (*Proc Natl Acad Sci USA*, 1999) showed that the majority of patients discontinuing effective potent therapy exhibited dramatic viral rebound. Patients who exhibited lower levels of viremia were those in whom treatment was started soon after primary infection. This accords well with data from a study by Rosenberg and colleagues (*Nature*, 2000) suggesting that very early treatment may alter the viral set-point. An explanation is that early treatment shuts down viral replication before a larger viral reservoir (which would result in higher replication with removal of drug treatment) can be established. A second factor that may limit the size of the viral reservoir is host genetic background. For example, although the significance of the finding remains unclear, accumulating data suggest that a majority of patients who are long-term nonprogressors with low viral load (<50 HIV-1 RNA copies/mL) off treatment exhibit the human leukocyte antigen (HLA)-B57 haplotype (Migueles et al, *Proc Natl Acad Sci USA*, 2000). Similarly, polymorphisms in chemokine receptors may play an important role (O'Brien et al, *Annu Rev Genet*, 2000).

With regard to the role of HIV-specific CD8+ T cells in determining viral set-point, there is every reason to believe that these cells are important to the control of HIV infection. They have been shown to be essential for viral control in a variety of animal model systems, and have been found to exhibit a range of effector functions, including cytolytic activity, when exposed to HIV or HIV antigens. However, their specific role in host immune control of HIV infection remains unclear. CD8+ T cells reactive to HIV antigens decrease in number during prolonged effective antiretroviral therapy; when therapy is stopped, cell number rapidly increases without necessarily leading to a spontaneous suppression of plasma HIV-1 RNA level. These findings suggest that although the increase in levels of HIV-specific CD8+ T cells in the peripheral blood on stopping therapy is virus-driven, this increase is not necessarily indicative of a response that is effective in controlling the virus. Given what is known about the activity of these cells, the findings also suggest that it is unlikely that the number of these cells in the peripheral blood reflects the total body activity of the cells.

Similarly, it is difficult to draw conclusions regarding the effects of HIV-specific CD4+ T cells on viral control on the basis of currently available data. Although scientists initially believed that HIV-specific CD4+ T cells were preferentially depleted in HIV infection, recent data suggest that at least some subsets of cells with specificity for HIV antigens persist in chronic infection—although the role they may play in viral control is uncertain. One recent study (McNeil et al, *Proc Natl Acad Sci USA*, 2001) has shown that CD4+ T-cell responses, as measured by the HIV p24 antigen-specific lymphoproliferative assay, which reflects responses in relatively resting cells, are more likely to be present in patients on potent antiretroviral therapy. Assays that measure p24-induced interferon-γ reflect more activated HIV-specific CD4+ T cells (Figure 4). Cessation of therapy results in increased HIV-1 RNA levels, decreased lymphoproliferative responses, and increased interferon-γ-producing responses. Consideration of both assay results suggests that HIV-specific CD4+ T cells are present but have shifted from a predominantly resting to a predominantly activated state with cessation of therapy. With resumption of therapy, lymphoproliferative response increases and interferon-γ-producing response decreases somewhat.

In this and other studies, it is consistently observed that the proliferative capacity of HIV-specific CD4+ T cells, as well as that of CD4+ cells with specificities for non-HIV antigens, is compromised in the setting of high levels of HIV viremia. However, the clinical significance of these findings is unclear—a point recently underscored by the observation that despite the successful induction of CD4+ T-cell responses to p24 antigen by exogenous administration of HIV antigens in infected patients, there is no significant change in plasma HIV-1 RNA levels or progression to AIDS-defining events (Kahn et al, JAMA, 2000).

One area of research to determine how host immune response may be manipulated to complement the success of antiretroviral therapy is that of

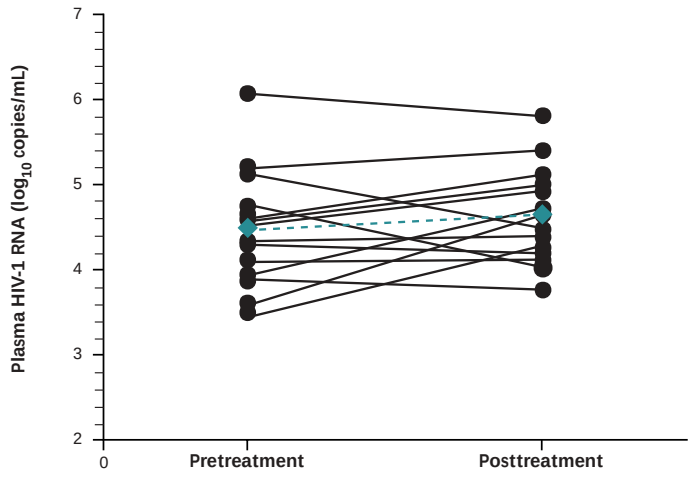

Figure **3.** Levels of plasma viremia before initiation of potent antiretroviral therapy and after cessation of therapy in 14 patients in whom plasma HIV-1 RNA level was maintained at lower than 50 copies/mL for 1 to 4 years. Solid black lines indicate patients; dashed green line indicates mean. Adapted with permission from Hatano et al, *AIDS,* 2000.

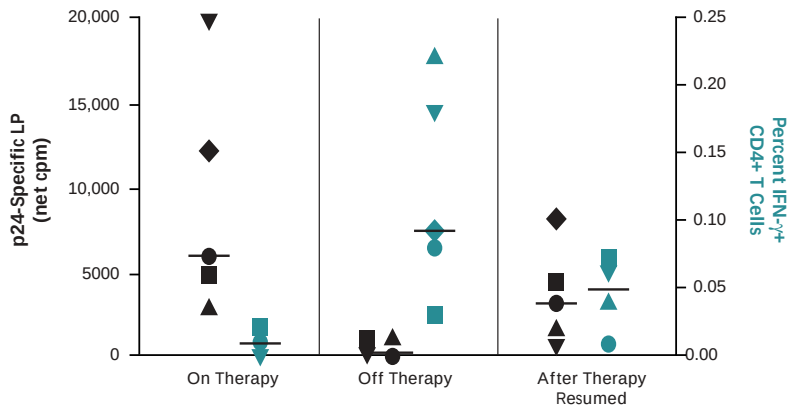

Figure **4.** Effect of potent antiretroviral therapy, cessation of therapy, and resumption of therapy on HIV-specific CD4+ T-cell lymphoproliferative (LP) response (p24-specific LP) and proportion of HIV-specific CD4+ T cells (percent interferon-γ-positive [IFN-γ+] cells); cpm indicates counts per minute. Adapted with permission from McNeil et al, *Proc Natl Acad Sci USA,* 2001.

immune-based therapies. Studies of the effects of a 5-day cycle of interleukin-2 treatment in HIV-infected patients using DNA labeling have shown that treatment increases the percentage of dividing CD4+ T cells and increases the half-life of the cells (Kovacs et al, *J Exp Med*, 2001). The potential clinical effects of the increase in CD4+ T-cell production and, more importantly, the prolongation of cell survival with interleukin-2 treatment are being investigated in 2 large international phase 3 trials (SILCAAT and ESPRIT).

Summary

The immune systems of patients with HIV infection are characterized by polyclonal activation and immunodeficiency. The magnitude of the polyclonal activation appears to be directly related to the level of HIV viremia. Potent antiretroviral therapy leads to immediate improvement in the degree of activation and in many of the clinical manifestations of HIV infection. The recovery of T-cell numbers is a slower process and is likely driven by peripheral expansion rather than thymic output. Although CD4+ and CD8+ T cells that respond to HIV antigens can be identified, their precise role in host defense against HIV remains unclear. The single best measure to evaluate host immune response to HIV remains the plasma HIV-1 RNA level in the absence of therapy.

Presented in March 2002. First draft prepared from transcripts by Matthew Stenger. Reviewed and updated by Dr Lane in June 2002.

Financial Disclosure: Dr Lane is co-inventor on the patent for the use of interleukin-2 in HIV infection held by the US government.

Suggested Reading

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World Health Organization Guidelines for Scaling Up Antiretroviral Therapy in Resource-Limited Settings

The World Health Organization (WHO) issued draft guidelines in April 2002 for the scaling up of antiretroviral therapy in developing countries. The guidelines, written by an international group of experts, are intended to lower the technical barriers to treatment by recommending standardized regimens and simplified monitoring in resource-limited settings. Although applicable to the clinical practices of individual health care practitioners, the authors state that the recommendations are designed for senior-level policymakers in these settings.

The document includes guidelines for when to initiate and switch antiretroviral therapy, regimens for treatment-naive and treatment-experienced patients, and treatment for pregnant women and children. Specific recommendations include:

- For adolescents and adults, antiretroviral therapy should be initiated based on WHO stage of HIV disease; for those without clinical AIDS, CD4+ cell count if available or total lymphocyte count. For children, antiretroviral therapy should be initiated based on WHO pediatric stage of HIV disease and, if possible, virologically proven infection (by HIV polymerase chain reaction, immune complex dissociated HIV p24 antigen detection, or HIV culture); for those without clinical AIDS or for whom virologic confirmation is not possible, CD4+ cell percentage.

- In adolescents and adults, the initial regimen should consist of a dual nucleoside reverse transcriptase inhibitor (nRTI) backbone plus 1 additional drug (ie, efavirenz or nevirapine, abacavir, or a ritonavir-boosted protease inhibitor or nelfinavir). Regimens consisting of 2 nRTIs only are not recommended. In children, 2 nRTIs and a nonnucleoside reverse transcriptase inhibitor or abacavir are recommended (although efavirenz for children younger than 3 years old is not recommended).

- For pregnant women and women with the potential to become pregnant, zidovudine, lamivudine, nevirapine, nelfinavir, or saquinavir/ritonavir are recommended as possible components of an antiretroviral regimen.

- Because viral load tests are not usually available in resource-limited settings, clinical and, where possible, CD4+ cell count criteria should be used to define treatment failure.

- When treatment failure occurs, all of the drugs in the current regimen should be switched, ideally to at least 3 new drugs with at least 1 drug from a new class. This recommendation recognizes the reality that drug resistance testing is not routinely available for individualized patient management in resource-limited settings.

- Countries that implement antiretroviral therapy programs are encouraged to consider monitoring for drug resistance on a population level as therapy is introduced on a broad scale. This can be done through participation in the recently announced WHO-International AIDS Society Global HIV Drug Resistance Monitoring Project.

- Laboratory tests are prioritized into 4 categories: "absolute minimum," "basic recommended," "desirable," and "optional." The absolute minimum tests are defined as an HIV antibody test and a hemoglobin or hematocrit level. CD4+ count is included in the desirable category and viral load testing in the optional category. The need for low-cost, widely available laboratory assays for CD4+ cell and viral load monitoring is emphasized.

The WHO estimates that at least 6 million people in the developing world currently need antiretroviral therapy, and that fewer than 5% of those have access to treatment. The organization proposes that by the end of 2005, 3 million people should be receiving antiretroviral therapy.

The document, "Scaling Up Antiretroviral Therapy in Resource-Limited Settings: Guidelines for a Public Health Approach," is available online at:

www.who.int/HIV_AIDS

Copies may also be ordered by e-mail request to hivaids@who.int. For the most recent treatment recommendations by the International AIDS Society–USA, see the insert in this issue of *Topics in HIV Medicine* or visit www.iasusa.org for a link to *JAMA*.