

Perspective

Pathogenesis of HIV Infection: Total CD4+ T-Cell Pool, Immune Activation, and Inflammation

Recent studies have yielded important findings on the pathogenesis of HIV infection. HIV infection leads to immune dysfunction through CD4+ T-cell depletion (immunodeficiency) and immune activation (immunosuppression). In vivo imaging studies of nonhuman primates indicate that the total body pool of CD4+ T cells may provide more accurate quantitation of immune depletion in HIV infection than the peripheral blood CD4+ count. Immune activation appears to be driven by both a homeostatic response to CD4+ cell depletion and an inflammatory response to HIV infection. The evidence is mounting that ongoing inflammation and coagulation account for the increased risk of serious nonopportunistic events in patients with HIV infection. Studies in long-term nonprogressors indicate that the HIV-specific immune responses in these patients are distinguished by clonal expansions of antigen-specific CD8+ T cells. Additional study of the precise mechanisms that allow immunologic control of infection in these patients may contribute to development of vaccines and immune-based therapies. This article summarizes a presentation made by H. Clifford Lane, MD, at the International AIDS Society–USA continuing medical education program held in May 2009 in Chicago. The original presentation is available as a Webcast at www.iasusa.org.

The pathogenesis of HIV infection includes depletion of the total body CD4+ T-cell pool, leading to immunodeficiency. This effect is accompanied by activation of numerous elements of the immune system, leading to a functional immunosuppression (ie, reduced function of remaining CD4+ cells) and a state of inflammation and coagulation that appears to underlie the increased risk of nonopportunistic complications observed in patients with HIV infection. Inadequate immune response to HIV infection allows ongoing viral replication, driving continued immune activation. Recent investigations have focused on clarifying the relationship between peripheral blood CD4+ T-cell count and the total body CD4+ T-cell pool, dissecting the role of immune activation and inflammation in the expanded spectrum of complications in HIV disease, and identifying potential correlates of protective immunity to HIV.

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Characterization of Total CD4+ T-Cell Pool: In Vivo Imaging

The correlation between peripheral blood CD4+ counts and the spectrum of clinical manifestations of HIV disease, from generalized lymphadenopathy at higher counts to cytomegalovirus retinitis at low counts, is well defined and has been recognized for many years. More recently, an association has been shown between peripheral blood CD4+ count and risk of nonopportunistic morbidity and mortality. For example, the CASCADE (Concerted Action on Seroconversion to AIDS and Death in Europe) study in treatment-naive patients and the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study in treatment-naive and treatment-experienced patients showed progressive increases in risk of all-cause mortality and non-AIDS-related mortality with decreasing CD4+ counts.

Despite its value, the peripheral blood CD4+ count is recognized as an imperfect marker of HIV disease progression. Baseline CD4+ counts ex-

plain only up to 30% of the variability in the time to reach AIDS or death (Mellors et al, *JAMA*, 2007). In patients who have undergone splenectomy or have received marrow-suppressive therapies such as interferon-alpha (IFN- α), the CD4+ percentage is a better marker of immune competence than CD4+ count. Further, given that only a small percentage (estimated at 2%) of the total pool of CD4+ cells are present in the blood at any moment in time, even a small change in the distribution of cells between the lymphoid tissues and blood could result in a large change in the peripheral blood CD4+ count.

Studies have recently been undertaken in the nonhuman primate simian immunodeficiency virus (SIV) model of HIV infection to better understand the relationships between the peripheral CD4+ count and the total CD4+ cell count in health and during SIV infection. In part, this interest was spurred by repeated statements at scientific meetings that approximately 70% of CD4+ cells are located in the gut. This figure seemed high and was difficult to validate with published experimental data.

To study the total CD4+ cell pool in nonhuman primates, a series of imaging studies were performed. A nondepleting humanized monoclonal antibody that binds to CD4 molecules from humans and rhesus macaques (CDR-OKT4A/hlgG4 [OKT4A]) was conjugated with indium 111 (¹¹¹In) in a manner similar to that used by Rubin and colleagues in 1996 to study CD4+ T-cell distribution in mice (*Proc Natl Acad Sci USA*, 1996). Semiquantitative in vivo images of CD4+ cell distribution were obtained using a single-photon emission computed tomography (SPECT) camera with normalization against liver uptake. Quantitative assessment of in vivo tissue binding by the labeled antibody was performed by ex vivo analysis of tissue in a gamma counter.

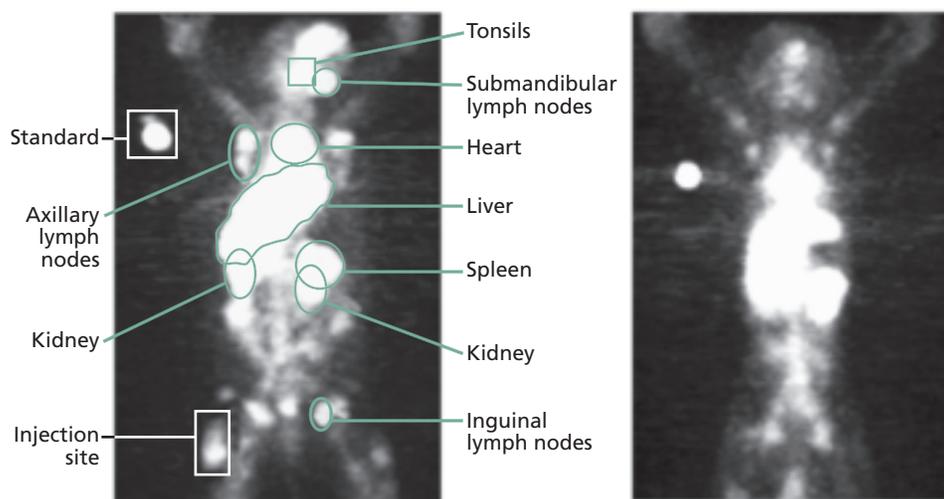


Figure 1. Total body imaging of CD4+ T-cell pool in 2 uninfected macaques, using indium 111-labelled anti-CD4 antibody. CD4+ counts: left, 1700 cells/ μ L; right, 1378 cells/ μ L. Adapted from Di Mascio et al, *Blood*, 2009.

Radioactivity was normalized against activity in the liver.

Macaques injected with ^{111}In -labeled antibody showed binding of the anti-CD4 antibody to CD4+ cells in vivo. A 10- to 20-fold increase in antibody binding was observed for CD4+ cells compared with CD4- cells derived from spleen, axillary lymph nodes, and mesenteric lymph nodes. Total body imaging of the CD4+ cell pool in uninfected monkeys with normal CD4+ counts showed that uptake of the antibody was highest in the lymph nodes, tonsils, spleen, blood pool of the heart, and liver (Figure 1). Studies of animals with SIV or simian-human immunodeficiency virus (SHIV) infection showed that the intensity of anti-CD4 antibody staining in lymphoid tissues (spleen, tonsils, lymph nodes) but not in non-lymphoid tissue (heart, kidney, marrow, testes) was proportional to the peripheral blood CD4+ count (Figure 2, A). Plots of the peripheral blood CD4+ counts versus radiotracer retention in lymphoid tissue showed an exponential relationship (Figure 2, C).

Thus, decreases in peripheral CD4+ count at relatively low counts are correlated with larger decreases in the total CD4+ cell pool than similar absolute decreases at higher CD4+ counts. The reduction in the total CD4+ pool is quite large as the peripheral cell count decreases below 200 cells/ μ L, an obser-

vation that fits well with the dramatic increase in risk of opportunistic conditions as peripheral cell counts decline and continue to fall below this value.

Of note, the density of CD4+ T cells in intestinal tissue appeared to be low. The gastrointestinal tract appeared to account for no more of the CD4+ pool than the spleen.

Immune Activation

T-cell activation can be measured in several ways. Proliferation can be assessed using an antibody to measure incorporation of bromodeoxyuridine (BrdU) into the DNA of cells during cell division (S phase of the cell cycle) and by measuring the reduction of intensity of staining with fluorescent carboxyfluorescein diacetate succinimidyl ester (CFSE), a dye that stains cell cytoskeleton and thus decreases by 50% with each cell division. Nonproliferation-based assessments of activation include measuring expression of cell surface markers such as HLA-DR, CD38, and programmed death-1 (PD-1) or measurements of intracellular proteins such as Ki67 and IFN- γ .

Immune activation in HIV infection includes 2 components: (1) the homeostatic response to CD4+ cell depletion, a compensatory mechanism that may include production of interleukin-7 (IL-7); and (2) an inflammatory

response that includes both an HIV-specific immune response and “by-stander” immune activation as a result of the HIV-specific immune response. In HIV infection, CD4+ cell activation and CD8+ cell activation are regulated differently, reflecting the different influences of these 2 forces on CD4+ and CD8+ T cells.

In a study in which T-cell proliferation was measured by BrdU incorporation, HIV-infected patients were categorized into 4 groups according to viral load and CD4+ count. The level of CD8+ cell activation was correlated almost exclusively with viral load (Figure 3). In contrast, the level of CD4+ cell activation was associated with both viral load and CD4+ cell depletion. The highest levels of CD4+ and CD8+ activation were in the subset of patients with high viral loads and low CD4+ counts. The second highest level of CD4+ activation occurred in patients with low CD4+ counts and low viral loads, whereas the second highest levels of CD8+ activation were in patients with high viral loads and high CD4+ counts.

The differences in activation of CD4+ and CD8+ cells are also evident from a study in patients with well-controlled viral loads (plasma HIV RNA levels < 50 copies/mL). As can be seen from Figure 3 (bottom), the level of CD4+ cell activation (BrdU incorporation) in HIV-infected individuals decreases as the CD4+ count increases and eventually becomes indistinguishable from that of HIV-uninfected subjects (Catalfamo et al, *Proc Natl Acad Sci USA*, 2008). Conversely, CD8+ cell activation in the HIV-infected group was always greater than that in the uninfected group. This persistent increase in CD8+ cell activation likely represents ongoing viral replication, even in patients with plasma HIV RNA levels below 50 copies/mL.

Inflammation as a Component of HIV Immune Activation

Patients with HIV infection are at increased risk of morbidity and mortality from a variety of nonopportunistic serious conditions, and it has become

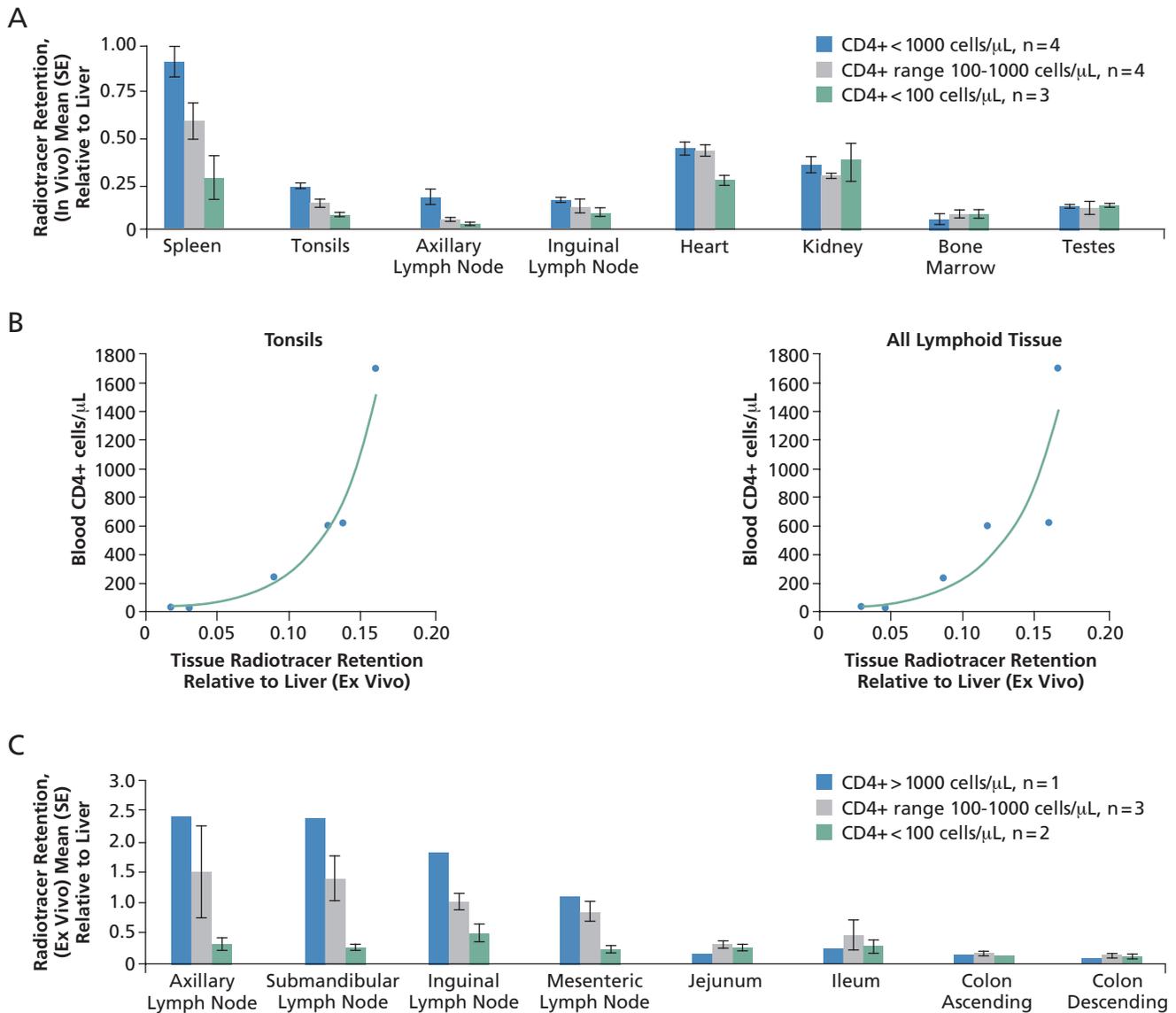


Figure 2. Data from simian immunodeficiency virus- or simian-human immunodeficiency virus-infected and uninfected macaques, showing that intensity of in vivo staining of CD4+ cells in lymphoid tissues (A) was exponentially proportional to peripheral blood CD4+ counts (B). SE indicates standard error. Data from ex vivo tissue studies (C) showing density of CD4+ cells in lymphoid tissue and intestinal tissue according to peripheral blood CD4+ count. Adapted from Di Mascio et al, *Blood*, 2009.

increasingly clear that inflammation contributes to this increased risk. The SMART (Strategies for Management of Antiretroviral Therapy) trial compared strategies of drug conservation (intermittent antiretroviral therapy to maintain CD4+ counts above 250 cells/μL) versus continuous viral suppression with antiretroviral therapy. The goal of this trial was to determine whether reducing overall antiretroviral drug exposure might reduce the risk of non-AIDS-related complications potentially

due to antiretroviral drugs such as heart disease, stroke, and liver failure. The study was stopped prematurely when it was found that patients in the drug-conservation group had a higher risk of non-AIDS-related events than did those in the continuous-treatment group, with a hazard ratio of 1.7 for all serious non-AIDS-related events.

These findings suggested that the increased risk of non-AIDS-related events was associated more with HIV replication than with drug toxicity. A

subsequent analysis of biomarkers from stored samples showed that the risk of all-cause mortality was statistically significantly greater among patients with higher baseline levels of high-sensitivity C-reactive protein (adjusted odds ratio [OR], 2.8; $P = .03$); interleukin-6 (IL-6) (adjusted OR, 11.8; $P < .0001$); or the coagulation marker D-dimer (adjusted OR, 26.5; $P < .0001$) (Kuller et al, *PLoS Medicine*, 2008). In patients receiving intermittent antiretroviral therapy, increases in D-dimer

levels were statistically significantly associated with the increases in viral load at 1 month after stopping therapy.

These findings indicate that ongoing inflammation and a procoagulant state may underlie the increased risk of non-AIDS-related events observed in patients with HIV infection. The elevation of D-dimer levels may be particularly noteworthy because it suggests that ongoing coagulation in the context of ongoing inflammation may lead to small-vessel damage and ultimately to end-organ damage.

Host Control of HIV Infection

Understanding how some patients are able to spontaneously control HIV infection remains one of the great challenges in HIV science. In most infected individuals, HIV replication continues despite a high frequency (10%–40%) of very broad (15–20 epitopes) CD8+ cell responses to autologous virus. That is, the CD8+ cells respond to viral antigens, but the response is inadequate to control infection. The exceptions are a group of patients who maintain plasma HIV RNA levels below 50 copies/mL and normal CD4+ counts in the absence of antiretroviral therapy. Even in these patients, however, there is evidence of the presence of virus, with proviral DNA and antibody to HIV found in all and HIV RNA detected in most. This long-term nonprogressor phenotype is highly associated with certain HLA genotypes, particularly the HLA-B*5701 genotype. Achieving an understanding of the precise immunologic mechanisms leading to this control is of great importance to the development of vaccines and immune-based therapies.

A recent study assessed CD8+ cell profiles in long-term nonprogressors, patients with viremic progression, and patients with HIV RNA levels of less than 50 copies/mL who were receiving antiretroviral therapy (Migueles et al, *Immunity*, 2008; Migueles et al, *Nat Immunol*, 2002). Long-term nonprogressors did not differ from viremic progressors in the percentage of their CD8+ cells responding to viral antigen by producing IFN- γ (Figure 4). However,

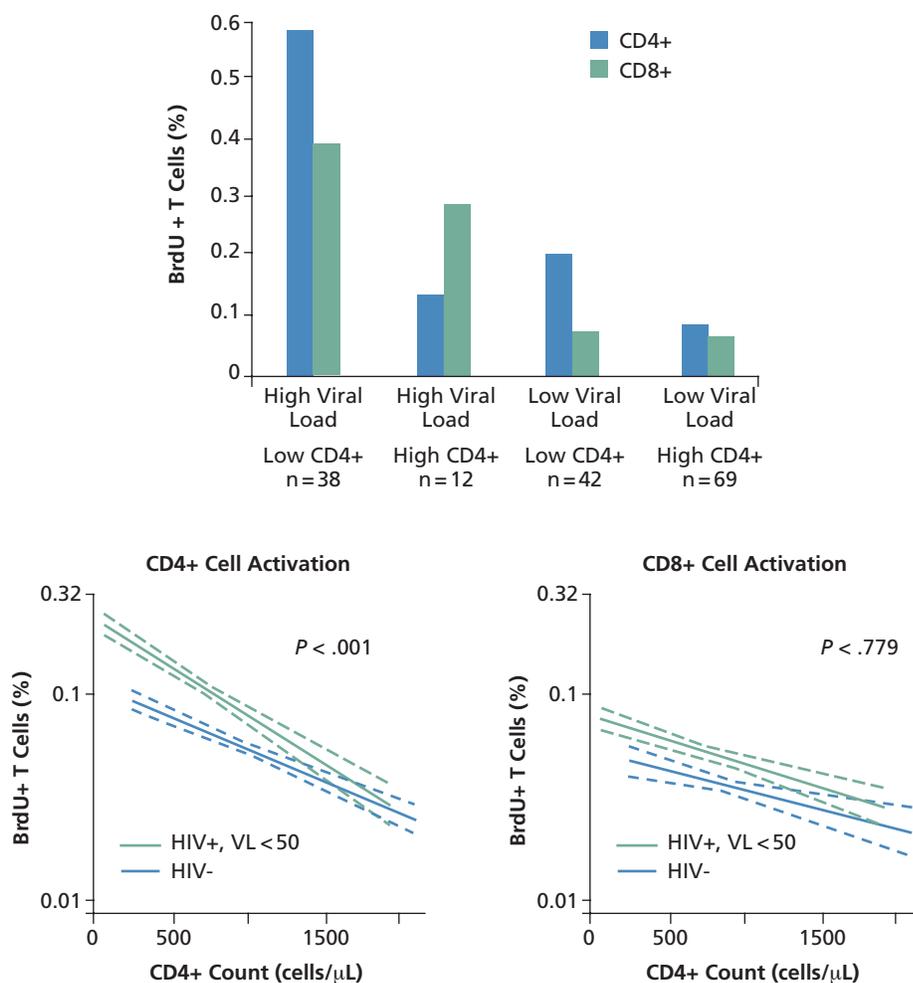


Figure 3. Top, CD4+ and CD8+ cell activation (as measured by bromodeoxyuridine [BrdU] incorporation) according to viral load and CD4+ count in HIV-infected patients. Bottom, CD4+ and CD8+ cell activation according to CD4+ count in HIV-infected (HIV+) patients with plasma HIV RNA level (VL) < 50 copies/mL and in uninfected (HIV-) subjects. Dashed lines indicate 95% confidence intervals; P values reflect the differences between the slopes of the 2 lines in each graph. Adapted from Catalfamo et al, *Proc Natl Acad Sci USA*, 2008.

proliferation of CD8+ cells, measured by CFSE staining, was statistically significantly greater in long-term nonprogressors than in either viremic progressors or patients with viral suppression who were receiving antiretroviral therapy. This finding suggests that a distinguishing immunologic feature in the long-term nonprogressors is the clonal expansion of activated cells in response to antigen. The cells undergoing clonal expansion have also been found to express perforin and granzyme B, key components of the cellular machinery activated during cell-mediated killing.

At the National Institutes of Health (NIH), a study is being conducted to de-

termine whether or not transfer of cells from a long-term nonprogressor to an HLA-matched progressor can confer protective immunity to HIV. The study plan involves obtaining peripheral blood mononuclear cells from an HLA-B*5701-positive long-term nonprogressor donor (plasma HIV RNA level < 50 copies/mL; CD4+ count \geq 400 cells/ μ L; no antiretroviral drugs for \geq 1 year) and infusing those cells to an HLA-B*5701-positive patient with failure of at least 2 antiretroviral regimens, HIV RNA level above 10,000 copies/mL, and CD4+ count below 200 cells/ μ L. Readers are encouraged to e-mail the researchers at the NIH (caldararo@niaid.nih.gov)

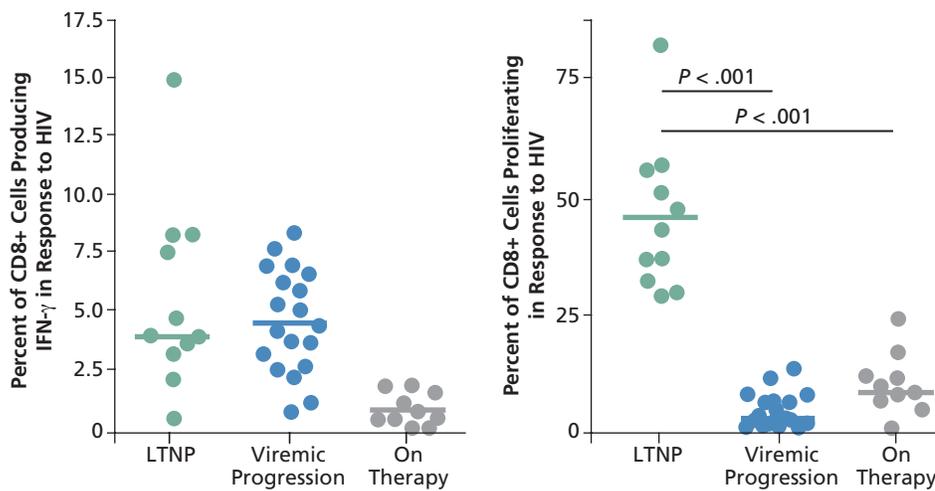


Figure 4. Percent of interferon-gamma (IFN- γ)-producing CD8+ cells and proliferation of CD8+ cells (measured by carboxyfluorescein diacetate succinimidyl ester staining) in long-term non-progressors (LTNP), viremic patients with progression, and patients with plasma HIV RNA level < 50 copies/mL who were receiving antiretroviral therapy. Left graph adapted from Migueles et al, *Immunity*, 2008; right graph adapted from Migueles et al, *Nat Immunol*, 2002.

should they know of a patient with potential interest in this study.

Summary

Recent investigations into the pathogenesis of HIV infection have yielded important insights. HIV infection leads to a depletion of the CD4+ T-cell pool that may be quantitated by in vivo imaging. There appears to be an exponential relationship between the peripheral blood CD4+ T-cell count and the total body CD4+ T-cell pool. The immune activation caused by HIV infection is driven by viral load and homeostatic forces and produces a state of chronic inflammation and coagula-

tion that may lead to substantial end-organ damage outside of the immune system. A small subset of patients with HIV infection exhibit control of viral replication in the absence of treatment. This control appears to be mediated via a unique CD8+ cell response that identifies an important target for potential HIV vaccines and HIV-specific, immune-based therapies.

Presented by Dr Lane in May 2009. First draft prepared from transcripts by Matthew Stenger. Reviewed and edited by Dr Lane in December 2009.

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Suggested Reading

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