

**Perspective****HIV Disease Progression: Immune Activation, Microbes, and a Leaky Gut**

*Recent findings indicate that the majority of all CD4+ T lymphocytes are lost during acute HIV infection, with mucosal compartments being most severely affected. The frequency of infection is very high in gut CD4+ T cells, and depletion of these cells persists into the chronic phase of infection. Infection is associated with increased gut permeability, with microbial translocation being evidenced by increased circulating lipopolysaccharide (LPS) levels. Plasma LPS levels correlate with systemic immune activation, which drives chronic HIV infection. Antiretroviral therapy reduces plasma LPS, and greater CD4+ T cell reconstitution is associated with lower LPS levels. These findings have a number of implications for therapeutic strategies. This article summarizes a presentation on HIV disease progression made by Daniel Douek, MD, PhD, at an International AIDS Society–USA Continuing Medical Education course in San Francisco in May 2007. The original presentation is available as a Webcast at [www.iasusa.org](http://www.iasusa.org).*

In its natural hosts (eg, sooty mangabeys, African green monkeys, chimpanzees), simian immunodeficiency virus (SIV) does not cause AIDS, other disease, or immunodeficiency, even in the presence of high viral loads. However, SIV infection in rhesus macaques—ie, an “unnatural” infection—results in rapid progression to AIDS, despite lower viral loads than in “natural” infection; similarly, SIV adapted to humans also causes rapid progression to AIDS. The mechanism for the progression to AIDS in HIV infection is depletion of CD4+ T lymphocytes, the target cells of the virus, with progression being clearly linked to a decrease in peripheral blood CD4+ cell numbers. In a series of studies investigators have sought to characterize how, where, and when HIV infection causes CD4+ T cell depletion, and to better understand how mechanisms underlying the disease process might differ in natural and unnatural infection.

**How, Where, and When?**

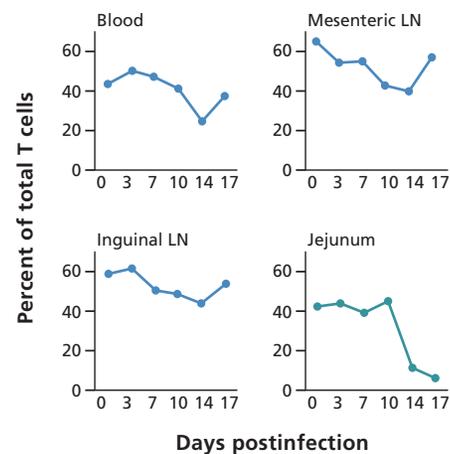
In the traditional view of HIV disease course, acute infection is accompanied

by a rapid transient decrease in peripheral blood CD4+ T cell count and a rapid partial recovery of this loss, with chronic infection being characterized by a gradual and profound decline in CD4+ T cell numbers. Thus HIV infection has been thought of as a relatively indolent disruption of CD4+ T cells eventually leading to collapse of immune function. This notion has been largely based on measurements of CD4+ T cell counts in peripheral blood.

In studies over the past several years, Dr Douek’s laboratory and others have shown that with HIV and in macaque and SIV infection, the earliest targets of infection are mucosal memory CD4+ T cells, which bear the CCR5 HIV coreceptor and which constitute the majority of CD4+ T cells. The greatest numbers of mucosal memory CD4+ T cells (indeed, the majority of all T cells) are found in the gastrointestinal (GI) tract, with this compartment harboring possibly 80% of the entire T cell population. In studies assessing mucosal CD4+ T cell depletion in acute macaque SIV infection in blood, mesenteric and inguinal lymph nodes, and the jejunum, loss was virtually complete in the GI tract within 17 days of infection, representing a profound loss in memory T cell population given the concentration of these cells in this compartment (see Figure 1). As can be seen by comparing the graphs for

peripheral blood with jejunum, neither the degree nor tempo of memory cell loss in the GI tract, the major reservoir of infected cells, could be predicted by the cell loss profile in the blood.

As shown in Figure 2, flow cytometric analysis in humans indicates an abundance of CCR5+ CD4+ T cells in the gut through which HIV could readily propagate. In a study in gut (terminal ileum) biopsies from more than 50 individuals with or without HIV infection, massive depletion of CCR5+ CD4+ T cells from the gut was found in HIV-infected patients (Figure 3, left). In HIV-infected patients, gut memory cells were more frequently infected than were peripheral blood memory cells, with the frequency differing by 10-, 100-, and occasionally as much as 1000-fold in individual comparisons (Figure 3, right). These findings, indicating both that the major reservoir of target and infected cells is the GI tract and that there is rapid and profound loss of cells in acute infection, provide a new model for HIV disease course (see Figure 4). On this model, the bulk of CD4+ T cell loss occurs within the first 2 to 3 weeks of acute infection.



**Figure 1.** CD4+ T cell depletion in peripheral blood, mesenteric lymph nodes (LN), inguinal LN, and jejunum after simian immunodeficiency virus infection of macaques.

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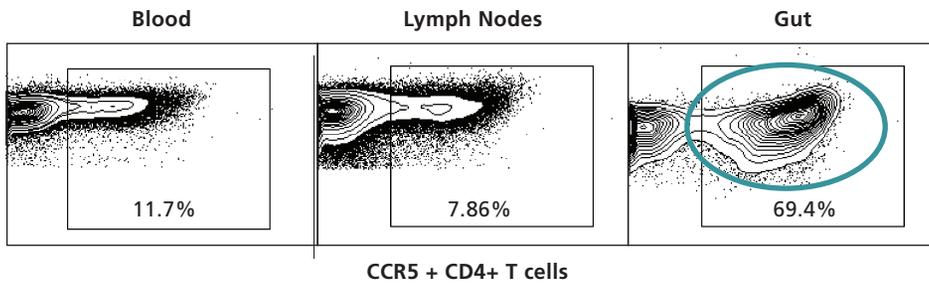


Figure 2. Flow cytometry results showing concentrations of CCR5+ CD4+ T cells in peripheral blood, lymph nodes, and gut in humans. There is an abundant supply of CCR5+ CD4+ T cells in gut through which HIV can rapidly propagate. Adapted from Brenchley et al, *J Exp Med*, 2004.

### Why Is HIV Disease Progressive?

The question then arises as to why there is progressive loss of CD4+ T cells beyond acute infection. To answer this question, aspects of immune activation in chronic HIV infection were investigated. Systemic immune activation has both beneficial and harmful effects in chronic infection and is in fact a strong predictor of disease progression, with recent findings indicating that it is a stronger predictor than peripheral plasma HIV RNA level. Immune activation in chronic HIV infection includes polyclonal B cell activation, increased turnover of T cells, a high frequency of “activated” phenotype T cells, and increased levels of cytokines, chemokines, and other proinflammatory mediators. The “good” effects of activation include restoration of memory CD4+ T cells and immunocompetence. The “bad” effects include lymph node fibrosis, retention of effector T cells in lymph nodes, thymic dysfunction, clonal exhaustion, drainage of memory T cell pools, and generation of more targets for HIV that permit ongoing HIV replication. What has been largely unclear is what is driving ongoing systemic immune activation in chronic HIV disease.

Given the massive depletion of memory T cells in the gut, microbial translocation from the gut was speculated to be involved in driving immune activation. In this process, gut-derived microbes or microbial products translocate to the systemic circulation in the absence of overt bacteremia. Microbial translocation is observed in

numerous settings, including graft versus host disease, inflammatory bowel disease, and gut surgery, and is correlated with systemic immune activation in some of these conditions. Enteropathy associated with HIV disease was initially reported as early as 1984 (Kotler et al, *Ann Intern Med*, 1984), with a number of other studies in subsequent years showing the presence of enteropathy, malabsorption, and increased intestinal permeability. It is now recognized that individuals with HIV infection can have the greatest increase in gut permeability among many of the GI epithelial barrier pathologies. Thus, both immunologic and structural defects in the GI tract in HIV infection can contribute to microbial translocation.

Microbial translocation can be quantified by measuring plasma levels of lipopolysaccharide (LPS; ie,

endotoxin). Plasma LPS levels were measured in approximately 300 subjects without HIV infection or with acute or early HIV infection, chronic infection, or AIDS (on the basis of CD4+ cell count <200/ $\mu$ L), with none of the subjects having any evident active infections other than HIV infection. Plasma LPS levels in HIV-seronegative subjects were similar to those in patients with acute or early HIV infection; however, patients with chronic HIV infection and those with AIDS (together termed “progressors”) had statistically significantly higher LPS levels than either HIV-seronegative subjects ( $P < .0001$  for both) or patients with acute or early HIV infection ( $P < .0001$  for both). A prior study by Suffedini and colleagues in which noninfected volunteers received injections of LPS showed that estimated plasma levels of as low as 14 pg/mL produced systemic immune activation measured as increased levels of inflammatory cytokines such as tumor necrosis factor, interleukin (IL)-1 receptor antagonist, IL-6, and IL-8 (Suffedini et al, *J Infect Dis*, 1999). In Dr Douek’s study, the median plasma LPS level in patients with progressors was 75 pg/mL, sufficient to stimulate systemic immune activation. That the source of circulating plasma LPS in HIV-infected individuals is predominantly the gut is suggested by studies showing dramatic reductions in LPS

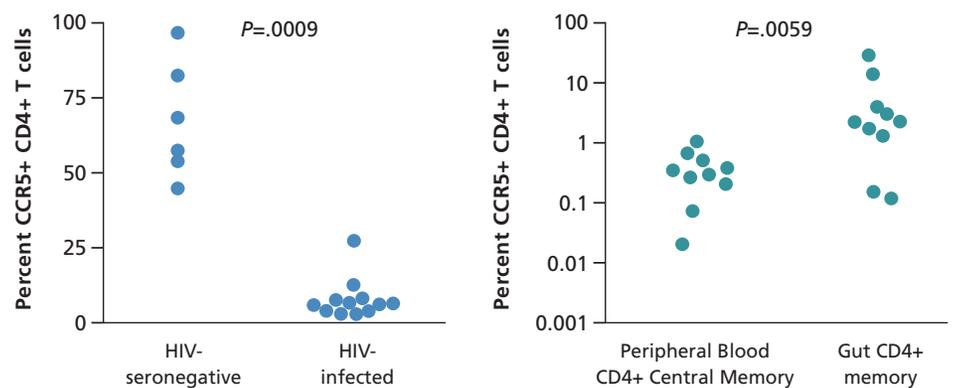


Figure 3. Left: Percentage CCR5+ CD4+ T cells in gut biopsies from HIV-seronegative and HIV-infected subjects. Right: Percentage of HIV-infected CD4+ T cells in the peripheral blood and in the gut. Gut biopsies are from more than 50 individuals. CCR5+ CD4+ T cells are massively depleted from the gut. There is a high frequency of infected cells at all stages of disease. Adapted from Brenchley et al, *J Exp Med*, 2004.

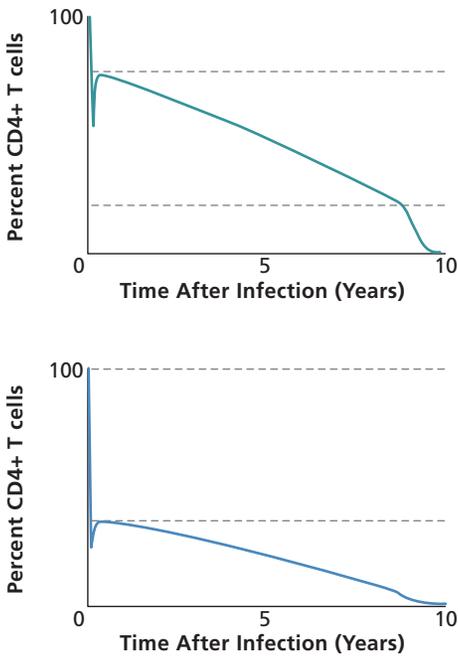


Figure 4. Top: “Old” view of HIV disease course. Blood CD4+ T cell counts: CD4+ T cell depletion occurs slowly over the course of the disease. Bottom: Revised view of HIV disease course, showing massive depletion of CD4+ T cells in acute infection.

levels in SIV-infected monkeys given large doses of “gut-sterilizing” antibiotics. Evidence that chronic LPS stimulation is occurring in HIV infection was then provided by studies showing substantially increased levels of soluble CD14 (sCD14) in plasma; LPS-stimulated monocytes secrete sCD14 and shed surface CD14. Both patients with acute or early HIV infection and those with progressive infection had markedly higher plasma sCD14 levels than HIV-seronegative subjects ( $P < .0001$  for both), indicating chronic in vivo LPS stimulation of monocytes and macrophages (Brenchley et al, *Nat Med*, 2006).

In these studies, LPS and LPS stimulation have been used as a marker for any immunostimulatory product that might be translocated from the gut to the circulation. Since LPS does not stimulate T cells directly, Dr Douek and colleagues investigated whether LPS levels correlate with other measures of non-LPS-mediated immune activation. An increased frequency of CD38+ HLA-DR+ CD8+ T cells is known to

occur in HIV infection and is associated with disease progression. It was found that the percentage of these cells correlated substantially with plasma LPS in the HIV-infected cohort (correlation [ $r$ ] = .5553;  $P = .0059$ ), indicating that other immunostimulatory factors are directly or indirectly activating these CD8+ T cells. Interferon alfa (IFN- $\alpha$ ) is produced by dendritic cells, which also are not stimulated by LPS. It was found that plasma IFN- $\alpha$  levels also correlated substantially with plasma LPS level in the cohort ( $r = .6244$ ;  $P < .0001$ ), again indicating that other immunostimulatory factors apart from LPS are active in HIV infection.

It is known that immune activation decreases with potent antiretroviral therapy, although it shows a much slower decline than does HIV RNA level and remains elevated above normal for at least a year after starting treatment. In a group of 28 HIV-infected patients, 24 had statistically significant decreases in plasma LPS after 48 weeks of full HIV RNA suppression on initial antiretroviral therapy ( $P = .0107$  for change from baseline in entire group). However, levels in the HIV-infected patients remained substantially higher than those in HIV-seronegative subjects ( $P = .0026$ ). As shown in Figure 5, CD4+ T cell count change was inversely correlated with LPS level after 48 weeks in these antiretroviral-treated patients ( $r = -.4628$ ;  $P = .0151$ ), suggesting that improved mucosal immunity reduces microbial translocation.

As noted, “natural” SIV infection is nonpathogenic and is associated with low immune activation despite high viral load. To determine whether such “natural” infection is associated with reduced microbial translocation, plasma LPS was measured in uninfected and SIV-infected macaques and in uninfected and SIV-infected sooty mangabeys. Whereas SIV infection was associated with a statistically significant increase in LPS in macaques ( $P = .002$ ), there was no difference in levels between uninfected and SIV-infected sooty mangabeys ( $P = .975$ ), suggesting absence of microbial translocation in “natural” infection.

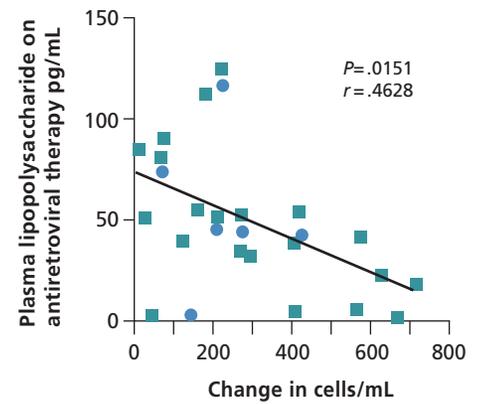


Figure 5. Inverse correlation of change in CD4+ T cell count and plasma lipopolysaccharide after 48 weeks of suppressive antiretroviral therapy. Immune activation decreases with antiretroviral therapy, but remains elevated above normal for at least 1 year. Adapted from Brenchley et al, *J Exp Med*, 2004.

## Summary and Implications

With HIV infection, the majority of all CD4+ T cells are lost during the acute phase of infection, with mucosal tissues being most severely affected. The frequency of infection is very high in gut CD4+ T cells, and depletion of these cells persists into the chronic phase of infection. Infection is associated with increased gut permeability and decreased enterocyte functionality, with increased circulating LPS levels indicating the occurrence of microbial translocation from the gut. Plasma LPS levels correlate with activation of innate and adaptive immunity in HIV infection, with suppressive antiretroviral therapy resulting in reduced plasma LPS levels and greater CD4+ T cell reconstitution being associated with reduced LPS levels. Measurement of LPS indicates that microbial translocation does not occur in nonpathogenic SIV infection.

These findings indicate that acute HIV infection is a very different disease state from chronic infection. Acute infection is characterized by massive and rapid CD4+ T cell loss, whereas chronic infection is characterized by persistent immune activation that drives viral replication and further CD4+ T cell depletion. The findings further indicate that the integrity of the

mucosal barrier is a paramount factor in disease progression.

Antiretroviral therapy is currently the best way to protect the gut and prevent microbial translocation and reduce chronic systemic immune activation. The ways in which these findings may alter our approach to treatment include perhaps changing the concept of “early” therapy to mean hours or days after exposure to HIV rather than weeks or months. Strategies for achieving early reduction of target cell infection need to be pursued—eg, by using microbicides applied mucosally. Further, pre-exposure and postexposure prophylaxis, if practically feasible, could be highly effective ways to prevent infection. Therapies to improve gut immune reconstitution (eg, cytokines) and to at-

tenuate mediators of inflammation (eg, antiseptics agents) could be pursued. In addition, there should be increased emphasis on the development of vaccines to prevent or reduce CD4+ T cell depletion at mucosal surfaces.

*Presented by Dr Douek in San Francisco in May 2007. First draft prepared from transcripts by Matthew Stenger. Reviewed and edited by Dr Douek in August 2007.*

*Dr Douek has no relevant financial affiliations to disclose.*

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### Suggested Reading

**Brenchley JM**, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med.* 2006; 12:1365-1371.

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