The Treatment of PHI (Part 2): Immune Augmentation Through STIs

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THE TREATMENT OF PATIENTS IN THE ACUTE STAGE OF HIV INFECTION has long been a contentious subject. While some data seem to indicate that the initiation of HAART during PHI is associated with few or no long-term advantages—which appears to have been the experience of Dr. Martin Markowitz and his colleagues at the Aaron Diamond AIDS Research Center (see page 16)—other research teams have gained extremely encouraging results, most notably Dr. Eric Rosenberg and his colleagues with the Partners AIDS Research Center at Massachusetts General Hospital (MGH). Not only has this research at MGH helped to define the immunologic significance of PHI, it has also led to a greater appreciation of the immune-boosting potential of antiretroviral therapy.

Host Immune Responses in HIV Infection

Upon entering the body via mucosal surfaces, dendritic cells transport HIV to lymphoid tissue-draining reproductive organs and oral and intestinal mucosal surfaces. From there, HIV is widely disseminated to other lymphoid organs, where it is passed on to cells migrating through the lymphoid tissues, its primary targets being CD4+ cells. Once viral RNA has been converted to proviral DNA and integrated into the cell's nucleus, new viral proteins are produced and assembled. At the same time, some viral proteins undergo a process known as proteolytic degradation. These proteins are then combined with class I major histocompatibility complex (MHC) molecules on the surface of the cell, which in turn signal the immune system, alerting it to the presence of a foreign invader.

Both cellular and humoral responses are then generated in response to HIV infection. Cytotoxic T-lymphocytes (CTLs) can kill infected cells—either by direct contact (cytolytic response) or through the release of soluble antiviral factors (non-cytolytic response) including RANTES, MIP-1α, and MIP-1β—before new virions are produced. However, this is possible only if the CTLs are present in the proper activation state and in sufficient number.

In long-term non-progressing patients (LTNPs)—loosely defined as a small percentage of the overall HIV-infected population with consistent low viral loads (usually less than 50 copies/mL), high CD4+ counts, and naive to antiretroviral therapy—Dr. Bruce Walker and his colleagues at MGH have consistently demonstrated the presence of robust and consistent CTL responses.

In most patients with progressive disease, there is also strong CTL function, at least during the earliest stages of infection. However, these responses decline with disease progression. Attempts to restore CTL responses—including a process in which CTLs were isolated from HIV-infected patients, stimulated *in vitro*, and reinfused into the patient—were largely unsuccessful, perhaps because these cells are dependent on antigen processing and, as a result, are unable to achieve HIV-specific activation (Yang, 1998). Moreover, in patients with progressive and end-stage disease, CTLs can be detected but are present in low numbers, again suggesting some impairment in function.

HIV-Specific Cellular Immune Responses

Many viral infections—including CMV, EBV, and HSV—are not eradicated from the body, but instead are completely contained by the immune system. Murine models of lymphocytic choriomeningitis virus (LCMV) may provide insight into the immune response of viral infections in humans: LCMV-specific CTLs are produced and maintained, ultimately keeping viremia in check (Butz, 1998). However, in situations in which CD4+ cells have been experimentally knocked out, CTL activity wanes over time and high levels of viremia ensue (Battegay, 1994; Matloubian, 1994).

These experiments suggest that there is a critical relationship between the presence of virus-specific CD4+ cells and the maintenance of an effective CTL response. In HIV infection, the ability of HIV-specific CD4+ cells to proliferate in response to HIV antigens is characteristically weak or absent, representing the most glaring defect in the immune system's repertoire against HIV. Evidence of this is discussed in great detail in a pivotal study published in *Science* by Dr. Eric Rosenberg and colleagues (Rosenberg, 1997).

According to the paper, several studies involving LTNPs have been conducted to test the importance of these HIV-specific CD4+ cell responses in controlling infection. Initial studies were performed in an HIV-infected hemophiliac with 18 years of documented infection, a normal CD4+ cell count, and a viral load less than 50 copies/mL, who had never been treated with antiretroviral drugs. Consistent with previous studies, an extremely vigorous CTL memory response was detected, with more than 1 HIV-specific CTL per 200 PBMCs. Vigorous CD4+ lymphocyte proliferative responses (LPR) were also present, as demonstrated by loss of activity with depletion of CD4+ cells.

Next, the association between viral load and LPR was studied in a cohort of 10 individuals with varying clinical histories and viral loads who had never been treated with antiretroviral therapy. The cross-sectional analysis demonstrated a highly significant inverse correlation: Individuals with the strongest p24-specific proliferative responses had the lowest viral loads, and those with higher HIV-RNA levels had a markedly decreased ability to respond to p24; analysis of CD4+ cell counts versus viral load showed only a trend.

To further characterize the HIV-specif-

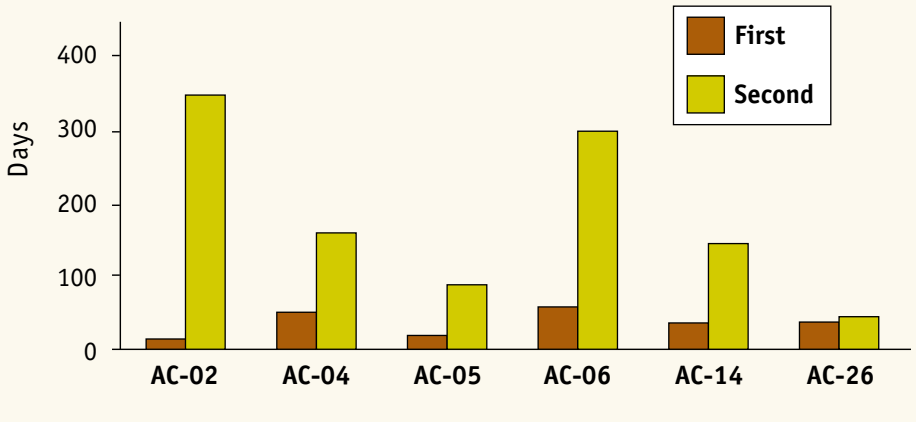
ic lymphocyte responses, Dr. Rosenberg's team examined the *in vitro* kinetics of LPRs over a six-day period in two LTNPs with robust responses. Both subjects demonstrated vigorous proliferative responses to both p24 and gp160, with detectable responses as early as day 2. In contrast, subjects with undetectable responses at day 6 likewise had undetectable responses at earlier times. Limiting dilution analysis revealed greater than 1 per 10,000 PBMCs for one subject and greater than 1 per 19,000 PBMCs for the second. As explained in Dr. Rosenberg's paper, such responses were highly characteristic of Th1 subset activity, as stimulation with viral antigen resulted in lymphocyte production of interleukin-2, interferon-γ, RANTES, MIP-1α, and MIP-1β, as opposed to the classic Th2 cytokines interleukin-4 and interleukin-10.

Acute Infection: The Time to Act

If HIV were like other viruses, acute infection should elicit a strong HIV-specific CD4+ cell activity, including robust LPRs to HIV antigens and, ultimately, broad-scale activation of CTLs. Yet, because HIV selectively infects activated CD4+ cells, it is believed that these cells become preferential targets for infection during the period when viral load is at its highest. Loss or dysfunction of these cells would ultimately impair immunologic activity needed to keep viral replication at bay, including activation of CTLs. The key then is to protect HIV-specific CD4+ cells during PHI, thereby augmenting their ability to respond to HIV and to maintain sufficient CTL activity.

There have, in fact, been a handful of studies indicating that HIV-specific CD4+ cell responses are maintained in patients receiving HAART during PHI. In a recent paper published in *Nature*, Dr. Rosenberg's team treated 16 patients with symptomatic PHI and two patients classified as being recently infected (infected for less than six months) (Rosenberg, 2000). With continued HAART, 15 patients achieved undetectable HIV-RNA levels (<50 copies/mL). All of these patients were found to have detectable HIV-specific CD4+ cell activity—on a par with LTNPs—a significant finding in light of the minimal HIV-specific CD4+ responses seen in untreated patients with acute infection and treated patients in the chronic phase of HIV disease.

FIGURE 1. Duration Off Therapy: First vs. Second Interruption



Source: Marcus Altfeld, MD

Compared to the first STI by 14 newly infected patients receiving HAART at Massachusetts General Hospital, the duration of time before treatment needed to be restarted — the criteria for restarting treatment included a viral load greater than 5,000 copies/mL for three consecutive weeks or if viral load exceeded 50,000 copies/mL at any one time — was significantly prolonged during the second STI. The differences in the duration off therapy in six patients who have completed two STI cycles are illustrated here.

While these data lent credence to the hypothesis that HIV-specific CD4+ cell responses can be preserved when treatment is started during acute HIV infection, they do not address the most clinically relevant question: whether or not these *in vitro* responses are associated with enhanced immunologic control of HIV replication *in vivo*. This, in turn, required a new study, one in which patients initiating HAART during the acute stage of HIV infection undergo a series of structured treatment interruptions (STIs) to determine how well HIV-RNA is controlled upon ceasing therapy.

Results from a preliminary STI study conducted by Dr. Rosenberg's team were reported two years ago (Rosenberg, 2000). Results of an expanded study conducted at Massachusetts General Hospital involving 14 patients were reported at the 8th Conference on Retroviruses and Opportunistic Infections, held last year in Chicago, by Dr. Walker (Walker, 2001).

All of the patients began treatment prior to, or at the time of, HIV seroconversion—they were all HIV antibody-negative and had high HIV-RNA levels-and had been receiving HAART for at least eight months before their first STI. As with the earlier eight-patient study, the plan was to restart treatment if their viral load exceeded 5,000 copies/mL for three consecutive weeks or if viral load exceeded 50,000 copies/mL at any one time.

During the first STI, HIV-RNA levels increased in all patients within 17 days, but levels in four patients soon dropped below 5,000 copies/mL. All four of these patients have remained off therapy, with two of them currently off treatment for almost two years and viral loads still below 500 copies/mL. Among the remaining patients, two additional patients were able to maintain viral loads below 5,000 copies/mL during the second STI and currently remain off therapy. The investigators noted that, compared to the first STI, the majority of patients experienced a much slower rebound in HIV-RNA levels during the second STI that would require them to restart therapy (see Figure 1). During the third STI, one additional patient experienced virologic control and has not yet been required to restart therapy. In total, seven of the 14 patients in this study appear to have maintained virologic control off therapy using STIs. The investigators are still conducting STI cycles in six of the remaining individuals, all of whom are currently nearing their second, third, or fourth STI. One patient has removed himself from the study but appears to have maintained a viral load of 7,500 HIV-RNA copies/mL after completing four STI cycles.

FIGURE 2A. Magnitude of CTL Responses

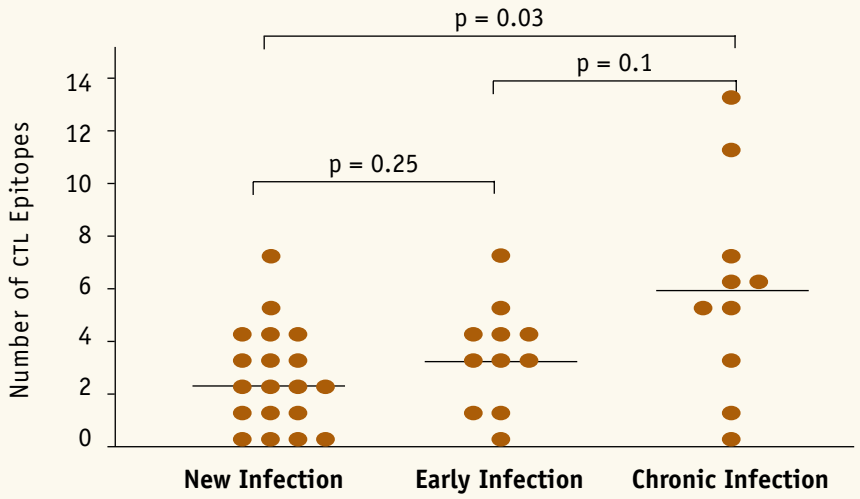
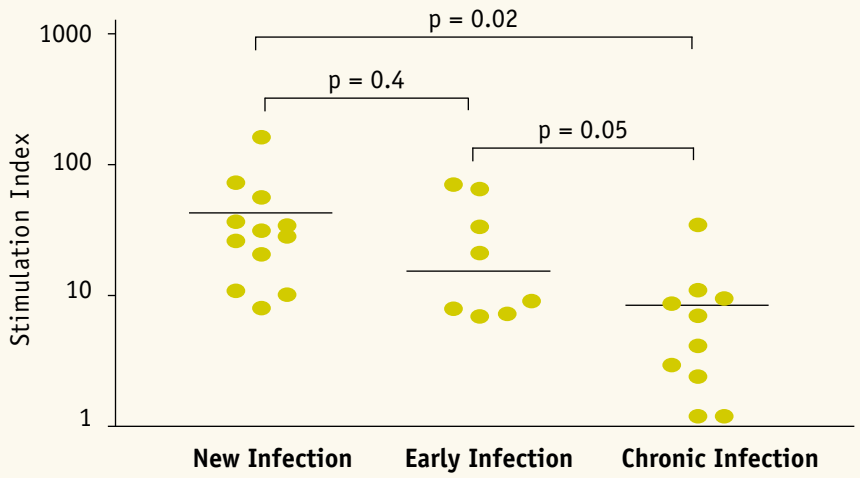


FIGURE 2B. Magnitude of HIV-Specific CD4+ Cell Responses



Source: Altfeld, 2001. Reprinted with permission of the *Journal of Experimental Medicine* and the Rockefeller University Press.

The CTL Response Mystery

Additional data reported by Dr. Walker show that HIV-specific CTL responses are actually weak and narrowly directed against a limited number of CTL epitopes in individuals treated during PHI. After all, the profound decline in HIV-RNA associated with HAART renders too little virus to effectively stimulate CTLs. However, CTL responses were significantly enhanced during the first and second STIs. But HIV-specific CTL responses have also been reported in a handful of studies involving chronically infected HIV-positive patients undergoing STIs. Unlike the results of the MGH study in individuals with treated acute HIV infection, the vast majority of chronically infected patients undergoing STIs have not been able to achieve durable virologic control. The disparity between these observations lies, perhaps, in a complex link between cellular immune responses and another factor that sometimes helps explain the unexplainable: viral diversity.

As discussed in a recent paper published in the *Journal of Experimental Medicine*, a research team led by Dr. Marcus Altfeld (also of Massachusetts General Hospital) examined HIV-specific CD4+ and CTL responses and viral genetic diversity in relation to duration of infection and subsequent response to antiretroviral therapy in two cohorts consisting of patients with acute HIV infection, patients with primary HIV infection (PHI), and chronically infected patients (Altfeld, 2001). The first cohort consisted of 30 patients divided into two groups: 19 who were diagnosed and treated with HAART before or at the time of HIV seroconversion (dubbed "newly infected" patients herein), and 11 treated with HAART after HIV seroconversion but within 180 days of primary HIV infection (dubbed "recently infected" patients). For comparison, a second cohort of ten individuals who were treated during chronic HIV infection was included in the analysis. These patients had been infected for at least 12 months before initiating a HAART regimen.

HIV-specific CTL responses were measured comprehensively using a panel of overlapping peptides spanning HIV and various CTL epitopes and were analyzed longitudinally in the newly infected patients and recently infected patients during the first year of antiretroviral therapy. Pretreatment HIV-specific CTL responses were of lower magnitude in the newly infected patients compared with the recently infected patients. However, the magnitude of HIV-specific CTL and CD4+ cell responses were characterized after a total treatment period of one year. In Figure 2A, the breadth of CTL responses is based on the number of HIV-specific CTL epitopes recognized in each patient after one year of treatment with HAART and is shown as a single dot. Breadth of CTL responses was compared between the patients with treated acute HIV infection (new infection; n=19), the patients who had seroconverted but had been infected with HIV for <180 days (early infection; n=11), and the patients who began treatment during chronic infection (chronic infection; n=10). The horizontal lines represent the median number of recognized CTL epitopes for each group. As shown here, the breadth of HIV-specific CTL responses during chronic infection were statistically higher and broader, compared to the breadth of responses seen in the patients with new and early HIV infection.

In Figure 2B, HIV-specific CD4+ cell responses in the same patients with new, early, and chronic HIV infection treated for one year are reported. As expected, patients initiating therapy during the primary stages of infection were more likely to generate HIV-specific CD4+ cell responses, compared to patients initiating therapy during the chronic stage of infection. The paradox of these data — it would be expected that more robust HIV-specific CD4+ cell responses during the primary stage of HIV infection would lead to more profound HIV-specific CTL responses, which was not corroborated here — can be explained by the proliferation of HIV quasispecies that essentially dilute CTL responses during chronic infection.

these responses in both groups increased significantly from baseline to two months of treatment. After 12 months of treatment, the mean CTL responses were comparable in both groups.

To compare the HIV-specific CTL responses in patients treated during early infection to those in patients treated during chronic infection, the breadth and magnitude of CTL responses were analyzed in the cohort of chronically infected patients, all of whom had been undergoing HAART for at least seven months. The mean HIV-specific CTL responses in these patients were significantly higher than those seen in either the newly infected or recently infected patients (see Figures 2A and 2B). Furthermore, CTLs in the chronically infected patients targeted a significantly greater number of HIV-specific epitopes than in the newly infected patients (average of 5.5 epitopes vs. 2 epitopes, respectively). The median breadth of CTL responses in the recently infected patients was three epitopes and was not statistically different from either the newly infected or chronically infected patients.

In a nutshell, CTL responses to HIV are generally weaker and more narrowly directed in newly infected patients, likely reflecting a relatively brief period of exposure to actively replicating virus and much higher and broader in patients with chronic infection. But this finding didn't make a whole lot of sense, given that HIV-specific CTLs are supposed to have an antiviral effect. What might explain this apparent paradox?

A number of research teams have suggested that HIV escapes from CTL-mediated pressure early after primary infection by the generation of escape mutants, ultimately requiring much broader CTL coverage to keep check on replication of a much more heterogeneous virus population. This, in turn, might explain where the immune response falls apart—a more concentrated CTL response against a homogeneous viral population may be more effective than a CTL response that spreads itself too thin to cover a highly diversified population of HIV.

In order to conduct an evaluation of the HIV genetic heterogeneity, Dr. Altfeld's team, in collaboration with Drs. Raj Shankarappa and James Mullins from the University of Washington, determined the variability of the C2-V5 region of the HIV envelope gene (env), using a heteroduplex mobility assay, in newly infected, recently infected, and chronically infected patients.

KEY POINTS

- HIV-specific cytotoxic T-lymphocytes (CTLs) are associated with long-term control of HIV replication. Vigorous HIV-specific CTL activity is seen in LTNPs, whereas more subdued CTL activity is seen in patients receiving HAART because of decreased antigen stimulation. In HIV-positive individuals with chronic infection who are not receiving HAART, HIV-specific CTLs are typically absent.
- HIV-specific CTLs are dependent on HIV-specific CD4+ cells. These cells are present during PHI, maintained in LTNPs, and lost in patients with progressive HIV disease. The initiation of HAART during PHI may reliably maintain HIV-specific CD4+ cells.
- Initiation of HAART during PHI can effectively maintain a population of HIV-specific CTLs. These CTL responses are usually of a low magnitude and are narrowly directed, likely because of the conservation of a homogeneous HIV population.
- The initiation of STIs after HIV has been maximally suppressed using HAART promotes the expansion and proliferation of HIV-specific CD4+ cells and the broadening of HIV-specific CTLs.
- STI strategies involving individuals treated during PHI may be more effective than STI strategies involving individuals who begin treatment during later stages of HIV infection. This is presumably because of the augmentation of HIV-specific CD4+ cell and CTL responses that are more focused on a limited viral population.
- STIs, involving both PHI-treated and chronically infected-treated patients, are still highly experimental. Clinicians are encouraged to refer individuals with PHI to an academic center focusing on the study of PHI and its treatment.

As reported in the team's *Journal of Experimental Medicine* paper, HIV env had very low levels of diversity in 6/7 newly infected patients, all of whom were highly adherent to their HAART regimens. The seventh individual in this group had a higher level of diversity, associated with poor adherence to his antiretroviral drug regimen, and had experienced several HIV-RNA rebounds during the first year of therapy. In contrast, the recently infected patients and the chronically infected patients showed significantly greater diversity in the env gene of the virus.

Taken together, these data indicate that the early initiation of HAART may conserve a very homogeneous virus population and lead to the development of strong HIV-specific CD4+ cell responses. Given the need for CD4+ cells to maintain effective CTL responses and the ability of virus diversification to accommodate immune escape, early therapy of primary HIV infection may be beneficial despite the induction of less robust CTL responses.

References

Altfeld M, Rosenberg ES, Shankarappa R, et al. **Cellular immune responses and viral diversity individuals treated during acute and early HIV-1 infection.** *J Exp Med* 193(2): 169-180, 2001.

Battegay M, Moskophidis D, Rahemtulla A, et al. **Enhanced establishment of a virus carrier state in adult CD4+ T-cell-deficient mice.** *J Virol* 68(7):4700-4, 1994.

Butz EA, Bevan MJ. **Massive expansion of antigen-specific CD8+ T cells during an acute virus infection.** *Immunity* 8(2):167-75, 1998.

Matloubian M, Concepcion RJ, Ahmed R, et al. **CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection.** *J Virol* 68(12):8056-63, 1994.

Rosenberg ES, Altfeld M, Poon SH, et al. **Immune control of HIV-1 after early treatment of acute infection.** *Nature* 407:523-526, 2000.

Rosenberg ES, Billingsley JM, Caliendo AM, et al. **Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia.** *Science* 278:1447-50, 1997.

Walker B. **State of the Art Lecture and Summary** [Abstract S37]. 8th Conference on Retroviruses and Opportunisitic Infections, Chicago, 2001.

Yang O, Wilkes B, Kalams SA, et al. Inhibition of HIV-1 by CD8+ cells from seropositive individuals is antigen processing-dependent [Abstract 60414]. 12th World Conference on AIDS, Geneva, 1998.