

HIGHLIGHTS

8th Conference on Retroviruses & Opportunistic Infections Chicago, February 5-10, 2001

Kendall A. Smith

- **Overview**

This conference will probably go down in history as a major turning point in the HIV epidemic. The NIH announced that it is changing the recommendations for the treatment of HIV infection from treating aggressively with Highly Active Anti-retroviral Therapy (HAART) as soon as the diagnosis is established, to a recommendation of waiting to initiate therapy until after the circulating CD4+ T cell concentration falls below 350 cells/ μ L. This is a seismic shift in policy, and represents a total reversal of opinion as to the safety and efficacy of HAART. HAART is effective therapy for HIV infection and is capable of suppressing viral replication. However, now it is recognized officially that ***HAART cannot cure HIV infection.***

If HAART is effective in suppressing HIV replication, it could still represent a long-term solution. However, the most compelling additional reason that the NIH has changed the treatment recommendations is that continuous HAART results in serious long-term toxicities, including metabolic acidosis, diabetes, and hypercholesterolemia with premature arteriosclerotic cardiovascular disease. Accordingly, the treatment of HIV infection with HAART is now similar to the treatment of cancer with cytotoxic chemotherapy: both therapies are only transiently effective, and both are considerably toxic. In many respects HAART for HIV infection is comparable to steroid therapy for rheumatoid arthritis. Both therapies are initially very effective, yet their continuous application leads to such serious side effects that they cannot be taken in effective doses continuously.

This policy change also represents an admission that HIV is a chronic infection that very slowly damages the immune system. Now that everyone has experienced the recovery of patients who had very low CD4+ T cells after several years of no effective therapies, it is clear that if therapy is delayed, even for several years, individuals can still recover when HAART is initiated. Accordingly, the idea that the immune system is irreparably damaged upon chronic HIV infection is no longer tenable.

The new guidelines do not enunciate a policy for individuals who are currently on HAART with undetectable plasma HIV concentrations and CD4+ T cells within the normal range (i.e. 600-1200 cells/ μ L). However, by extrapolation it is difficult to recommend continuation of HAART for these individuals, given the increasing incidence of serious side effects with increasing duration of therapy.

In view of the increasing evidence that recently infected individuals and even chronically infected individuals have readily detectable host reactivity to HIV (see below), it appears likely that immune-based therapies (IBTs) should be explored in conjunction with HAART, with the aim to stimulate HIV-specific immune reactivity to the point where HAART could be discontinued and the immune system could effectively contain any residual HIV in a latent state. Essentially, this occurs upon the immune reaction to all of the members of the herpes virus family.

There were several reports given at the meeting that warrant special mention in regard to IBTs:

- Abstract #272 by M. Betts from Rick Koup's group (Dallas) and Louis Picker's group (Portland) reported on studies analyzing total HIV-specific CD4 and CD8 responses to stimulation by mixtures of peptides derived from the HIV genes.

As background, it is well known that there is a reversal of the CD4:CD8 ratio in untreated, chronic HIV infection, which results from a decrease of CD4+ T cells and an increase in CD8+ T cells. Generally, the concentration of CD8+ T cells doubles, from ~ 500 cells/ μ L to ~ 1000 cells/ μ L. Although it is known that the decrease in CD4+ T cells occurs as a consequence of infection by HIV, the reason that CD8+ T cells increase has not been explained.

In experimental animal viral infections, such as lymphocytic choriomeningitis virus (LCMV) in the mouse and simian immunodeficiency virus (SIV) in the monkey, it has now been proven using newly devised assays that there is a marked proliferative expansion of virus-specific CD8+ T cells. This information has revolutionized our view of how the immune system responds to infections by intracellular microbes such as viruses.

This report by Betts and colleagues, *for the first time* reveals that HIV is no exception, and that the immune system recognizes and reacts to persistent HIV infection in the same way that it does in all other viral infections that have been well studied, i.e. by a huge increase in the concentration of CD8+ T cells. CD8+ T cells act to control viral infections by direct cell-cell killing of infected cells, and by secreting antiviral cytokines such as interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α).

Betts et al. used the technique of activating lymphocytes with mixtures of overlapping peptides taken from the sequences of every HIV protein to get a "*thorough, accurate, quantitative analysis of the T cell response to all possible antigens of HIV*". After a short-term activation, cells were analyzed by flow cytometry for intracellular cytokine production. They found that all 23 patients analyzed had readily detectable CD8+ T cells that recognized HIV. Moreover, the percentage of cells positive varied from 2%-25%. Given the fact that the assay of intracellular IFN- γ production only detects ~ 50% of the actual antigen-reactive

cells, these data indicate that 5%-50% of the circulating CD8+ T cells are reacting to HIV.

Data from the CD4+ T cells were also noteworthy. Based upon lymphocyte proliferation assays (LPA), it has been reported that individuals infected chronically have no HIV-reactive CD4+ T cells. However, the LPA is an insensitive assay, and is known to underestimate the frequency of antigen-reactive T cells. Betts and co-workers found that 0.2%-1.5% of CD4+ T cells reacted to the HIV peptide mixtures. At this time it is not clear whether the lower frequency of CD4+ T cells compared with CD8+ T cells is unique to HIV or will be found in other viral infections as well.

These data reveal just how active the immune system is in persistent HIV infection, and they provide the rationale and the hope that IBTs can be used to augment this pre-existing immune reactivity.

- **Symposium on Structured Treatment Interruption (STI)**

Bruce Walker from Massachusetts General Hospital chaired this session, which also was accompanied by a poster session.

Marty Markowitz from The Aaron Diamond AIDS Research Center summarized their data on STI after successful HAART for individuals identified early after infection (abstract # 288). Subjects were diagnosed within a mean of 60 days of the first symptoms of HIV infection, received an average of 3 years of HAART, had undetectable VLs and had mean CD4+ T cells of 811 cells/ μ prior to STI. All 14 subjects experienced a relapse of viremia within 20 days (mean), with peak VLs at 39 days (5.6 weeks). However, all subjects but one then lowered the peak VL by an average of 1.45 \log_{10} to a lower plateau ("set point"), a change that was accompanied by detectable increases in HIV-specific immune reactivity.

F. Garcia from Barcelona reviewed the results of a study (abstract # 289) of subjects infected chronically where the control group was left untreated, while the experimental group received 1 year of HAART interrupted for 4 week intervals 3 times during the year. Both groups then were evaluated after 1 additional year without therapy. After a year off of HAART, the treated group had a significantly lower VL, and higher CD4+ T cell counts than the control, untreated group.

P. Hermans of Brussels reported on 10 patients who discontinued HAART after 12-40 months of treatment for early primary infection (abstract # 290). Four of these patients controlled viral replication: most significant, 3 patients have undetectable VLs (< 50 copies/mL) after 18, 20 and 40 months of follow-up.

L. Ruiz of Badalona, Spain reported on a collaborative study with investigators from Paris and Edinburgh (abstract # 291) of a randomized controlled trial of 26 subjects infected chronically before HAART initiation. After 2 years of HAART,

the control group continued HAART, while the other group underwent 3 STIs for 30 days each, 90 days apart. After 3 STIs, this group appeared to have an antiviral response, in that the viral set point was $\sim 1 \log_{10}$ lower after the 3rd STI, compared with the 1st and 2nd STI. In addition, the burst of viremia after each STI was accompanied by an increase in HIV-specific effector CD8⁺ T cells in 42% of the patients.

Bruce Walker (Boston) reviewed their data on STI in subjects treated with HAART early after infection. Their preliminary data were published in Science in September, 2000. They now have studied 14 individuals and their results are similar to those of the group from Brussels. After one STI, 6 of these subjects were able to control their plasma HIV concentrations to low levels, $< 5,000$ copies/mL, without any further treatment. In 7 of the 14 patients, the plasma HIV concentration increased to $> 50,000$ copies/mL rapidly after the 1st STI, so that their protocol called for an immediate re-initiation of HAART. However, subsequent to a 2nd STI, 5 of these 7 individuals controlled the plasma virus concentration to $< 5,000$ copies/mL, suggesting that repetitive exposure to the virus stimulated the capacity of the host defenses to control viral replication.

I presented our data on 14 chronically infected individuals who had recovered normal CD4⁺ T cell concentrations and had elevated circulating CD8⁺ T cells and NK cells after at least 3 months of HAART plus daily low dose IL2 therapy (abstract # 360). In this study, subjects continued IL2 therapy during the interruption of HAART. All subjects underwent a relapse of plasma HIV within a mean of 19 days (almost identical to the Markowitz data), and after a rapid increase in plasma HIV concentration for ~ 2 weeks (mean doubling time 1.6 days), the VL peaked after a mean of 41 days (5.9 weeks). Subsequently, the plasma VL decreased a mean of $1 \log_{10}$ over the next 2 weeks (similar to the 1.45 \log_{10} decrease obtained by Markowitz), and then reached a trough (set point). Coincident with the decline in plasma HIV concentration, there was a doubling of the circulating CD8⁺ T cells, while the concentration of CD4⁺ T cells underwent a transient, mean 24% decrease, and the concentration of NK cells remained unchanged.

6 individuals have undergone a second interruption of HAART. In 4 of these individuals, the peak VL was >10 -fold lower after the second treatment interruption compared with the first interruption. In addition, the trough plasma HIV concentration was significantly lower after the 2nd interruption as compared to the first interruption in 3 of 5 subjects where data are available thus far.

From these reports it is evident that upon interruption of HAART, a very characteristic viral relapse occurs, with the onset of viremia detectable within $\sim 2-3$ weeks, a peak plasma HIV concentration within 5-6 weeks, followed by a decline in VL over the next 2 weeks, reaching a trough or "viral set point" by 8 weeks from the time of the interruption of antiviral therapy. Accordingly, these viral relapse dynamics can be used as an *in vivo* assessment of host antiviral

reactivity, and the treatment interruption can be a *Diagnostic Treatment Interruption (DTI)*, which can be used to assess the efficacies of various IBTs.

The total area under the plasma HIV concentration curve for the 8-12 weeks after the DTI should provide for a very rapid and quantitative analysis of the capacity of an IBT to change the magnitude of the host response to HIV. This is especially important for the evaluation of IBTs that may target different cells within the immune system: the ultimate test for efficacy is the antiviral effect of the immune system, and the FDA has already approved all of the antiretroviral agents on the basis of an antiviral effect.

HAART Sparing Regimens

As already mentioned, HAART resembles both cytotoxic chemotherapy for cancer and steroid therapy for rheumatoid arthritis. Therefore, agents and regimens that permit the discontinuation of HAART, but the maintenance of the immune system, would be expected to be beneficial in the long-term treatment of HIV infection. The FDA has approved agents that are “steroid-sparing” in the treatment of several diseases, and consequently it might be anticipated that clinical trials designed to test the efficacies of IBTs by the duration that patients can remain off of HAART.

- *Symposium on Primary Infection*

Giuseppe Pantaleo (Lausanne) and Susan Little (San Diego) chaired a very interesting symposium on primary HIV infection.

G. Rizzardì from the Pantaleo group presented data from a study where they examined the hypothesis that the release of pro-inflammatory cytokines during primary HIV infection actually favors the spread of the virus. They treated 9 subjects with Cyclosporine A (which blocks cytokine production) for 8 weeks together with HAART. Subsequently, CSA was discontinued but HAART was continued.

The effect of this approach on the plasma HIV concentration and the concentration of circulating CD4⁺ T cells were compared with data from 29 subjects who were treated with HAART alone. The CSA treatment resulted in a rapid restoration of normal concentrations of CD4⁺ T cells, while the group receiving HAART alone had depressed cell concentrations over the course of several months. There was no difference in the rate of decline of the plasma HIV concentration between the 2 groups.

Other data were consistent with the idea that the increase in CD4⁺ T cells occurred as a consequence of redistribution from the lymph nodes rather than from proliferation. The investigators have tested the efficacy of this approach in 3 individuals by an STI, and thus far, the plasma HIV concentrations have remained

at low levels, but additional subjects and a greater duration off of HAART is necessary to draw any conclusions.

J. Lifson presented data from the NCI, The New England Primate Center, the NIAID, Beth Israel Deaconess Hospital, Centocor, Gilead, and the Institute for Advanced Study-Princeton on experiments with Rhesus macaques and SIV. If treated with antiviral therapy early during infection just until plasma virus became undetectable, macaques maintained undetectable plasma SIV after discontinuation of therapy, and resisted challenges with infective SIV doses. However, when depleted of CD8⁺ T cell with monoclonal antibodies, these animals rapidly became viremic. Upon return of the CD8⁺ T cells, plasma SIV concentrations declined to pre-treatment levels, including < 100 copies/mL. The investigators concluded that CD8⁺ T cells are indispensable for sustained host control of SIV infection.

J. Zaunders presented data from St. Vincent's Hospital, Sydney Australia, The University of San Diego, and the Burnham Institute, San Diego on studies of T cell apoptosis during primary HIV infection. There was an increase in apoptosis among CD8⁺ T cells with an activated phenotype, while there was no increase in apoptosis among CD4⁺ T cells. Evidence was presented consistent with the view that these activated CD8⁺T cells might be dying because of cytokine withdrawal apoptosis. Therefore, their data could not incriminate apoptosis as a cause of the large decreases in CD4⁺ T cells observed during primary infection. These data and those of the Panteleo group on the effect of CSA during primary infection suggest that there is sequestration of CD4⁺ T cells at the site of cytokine-inflamed lymph nodes, and that during primary infection there is not a massive destruction of CD4⁺ T cells.