Long-Term Follow-Up of HIV-Infected Individuals Who Have Significant Increases in CD4⁺ Cell Counts during Antiretroviral Therapy

Susan L. Koletar, Paige L. Williams, Julia Wu, J. Allen McCutchan, Susan E. Cohn, Robert L. Murphy, Howard M. Lederman, and Judith S. Currier for the AIDS Clinical Trials Group 362 Study Team

1Division of Infectious Diseases, Ohio State University, Columbus, Ohio; 2Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, Massachusetts; 3Division of Infectious Diseases, University of California–San Diego School of Medicine, San Diego, and 4University of California at Los Angeles, Los Angeles, California; 5Infectious Diseases Unit, University of Rochester Medical Center, Rochester, New York; 6Northwestern University, Chicago, Illinois; and 7Eudowood Division of Pediatric Allergy and Immunology, Johns Hopkins University School of Medicine, Baltimore, Maryland

Background. Descriptions of the durability and consequences of immune reconstitution in patients who start highly active antiretroviral therapy (HAART) while severely immunosuppressed are limited.

Methods. Patients with previous CD4⁺ cell counts <50 cells/mm³, all of whom had HAART-induced increases in CD4⁺ cell counts of ≥100 cells/mm³ on 2 separate occasions (measured sequentially at least 4 weeks apart), were enrolled in a prospective trial and observed every 16–32 weeks. Evaluations included assessments for new opportunistic complications, virologic (human immunodeficiency virus [HIV] RNA load) and immunologic (CD4⁺ cell count) responses, or death.

Results. The median follow-up duration for 612 subjects was 184 weeks (range, 8–216 weeks). The rate of increase in CD4⁺ cell counts was ∼5.9 cells/mm³ every 8 weeks, with the degree of increase associated with the baseline HIV RNA load (<500 vs. ≥500 copies/mL). Subsequent measurements of virologic suppression based on HIV RNA levels were also associated with predicted CD4⁺ cell responses. Thirty-three AIDS-defining illnesses were reported (1.75 events per 100 person-years of follow-up); 40% (14 cases) occurred with higher than expected CD4⁺ cell counts.

Conclusions. CD4⁺ cell count increases are related to virological control, with continuing increases seen in individuals who are immunosuppressed. Opportunistic illnesses and/or complications are infrequent but can occur at any time, even in patients who maintained an elevated CD4⁺ cell count.

HAART dramatically reduces HIV infection–related morbidity and mortality [1–4]. Decreases in the incidence of opportunistic complications are related, at least in part, to significant increases in CD4⁺ cell counts that occur as a result of HAART-controlled suppression of HIV replication. As a result, opportunistic infections manifest more commonly either as acute inflammatory syndromes soon after initiating HAART [5–8] or with atypical presentations [9–11]. Additionally, a number of studies have documented the safety of discontinuing primary prophylactic therapies for disseminated Mycobacterium avium complex (MAC) disease, Pneumocystis jiroveci (formerly carinii) pneumonia (PCP), and toxoplasmosis [12–19] and of discontinuing secondary prophylactic therapies for disseminated MAC [20], histoplasmosis [21], and cytomegalovirus retinitis [22] in HIV-infected individuals who have had HAART-induced increases in CD4⁺ cell counts.

Although improved immune function is expected in patients who fully suppress HIV viremia, immune restoration may not be complete [23–26] or durable, and its relationship to the control of viral replication appears to be complex. Moreover, the long-term immunologic and clinical benefits for HAART-treated persons with initially severe immunosuppression have not been assessed. This report examines the long-term clinical outcomes observed in HIV-infected individuals who were severely immunosuppressed before responding to HAART.
**PATIENTS AND METHODS**

HIV-infected subjects with a history of CD4+ T cell counts <50 cells/mm³, no documented previous MAC-positive culture of blood samples or specimens obtained from another usually sterile body site, and sustained, HAART-induced increases to >100 cells/mm³ in the CD4+ cell count on 2 separate occasions (measured sequentially at least 4 weeks apart) were enrolled from October 1997 through April 1999 in a prospective, placebo-controlled trial involving the discontinuation of MAC prophylaxis. The protocol and its successive amendments were approved by the institutional review boards at each participating AIDS Clinical Trials Group (ACTG) site; each subject provided written informed consent. The primary objective of the trial was realized and demonstrated that the rate of MAC infection is low among patients with HAART-induced increases in CD4+ cell counts who discontinue MAC prophylaxis [12]. Subsequently, subjects have continued to be observed every 16–32 weeks to evaluate the development of new opportunistic or metabolic complications, neurological impairment, virologic (HIV RNA load) and immunologic (CD4+ cell count) deterioration, or death. The study chairs (J.S.C. and S.L.K.) reviewed and corroborated individual clinical events and specific diagnoses using the Adult ACTG Criteria for Clinical Events. Data submitted to the ACTG data management center from October 1997 through May 2002 are included in the present analysis.

In this report, we focused on the occurrences and rates of AIDS-defining illnesses as well as changes in CD4+ cell counts and HIV RNA levels over time. Exact 95% CIs for rates of death and AIDS-defining events were calculated under a Poisson distribution, and Poisson regression models were fit to evaluate the significance of trends in incidences over calendar time. Cox proportional hazards models were used to evaluate the association between potential predictors at baseline (i.e., at the time of enrollment into the trial of MAC prophylaxis discontinuation) and either the risk of developing an AIDS-defining infection or the combined end point of AIDS progression or death. The baseline predictors considered were age, sex, lowest historical CD4+ cell count (<25 vs. >25 cells/mm³), baseline CD4+ cell count, baseline HIV-1 RNA load >500 copies/mL, Karnofsky score of =$80, duration of HAART before study entry, previous receipt of a protease inhibitor, adherence to therapy, and study treatment received. For the purpose of this analysis, subjects were defined as being nonadherent to therapy if they reported failure to take any doses of antiretroviral medications during the week before study entry.

Trends in CD4+ cell counts over time were summarized graphically by presenting the mean and 95% CIs of data from each scheduled visit week. A generalized estimating equation (GEE) repeated measures model was used to further evaluate trends in CD4+ cell counts, allowing adjustment for the correlation among repeated measures on the same subject over time [27]. To evaluate the relationship between trends in CD4+ cell counts and HIV RNA loads during follow-up, subjects were categorized into 4 strata on the basis of their baseline and

### Table 1. Initial AIDS-defining event during follow-up.

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of cases</th>
<th>Total</th>
<th>Subject had CD4+ cell count &gt;200 cells/mm³</th>
<th>Laboratory value at the time of AIDS-defining event</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td>Pneumocystis jiroveci (carinii) pneumonia</td>
<td></td>
<td></td>
<td></td>
<td>13–19</td>
</tr>
<tr>
<td>Confirmed</td>
<td>3</td>
<td>0</td>
<td>13–19</td>
<td>409,000–750,000</td>
</tr>
<tr>
<td>Probable</td>
<td>6</td>
<td>4</td>
<td>100–535</td>
<td>&gt;500 to 307,000</td>
</tr>
<tr>
<td>Cytomegalovirus disease</td>
<td>4</td>
<td>2</td>
<td>2–377</td>
<td>&lt;500 to 203,000</td>
</tr>
<tr>
<td>Esophageal candidiasis</td>
<td>4</td>
<td>2</td>
<td>51–475</td>
<td>&lt;500 to 403,000</td>
</tr>
<tr>
<td>Mycobacterium avium complex</td>
<td>3</td>
<td>1</td>
<td>33–306</td>
<td>&lt;500 to 604</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>3</td>
<td>3</td>
<td>237–845</td>
<td>&lt;500 to 23,000</td>
</tr>
<tr>
<td>HIV wasting</td>
<td>2</td>
<td>1</td>
<td>187–225</td>
<td>&lt;500 to 42,000</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>2</td>
<td>0</td>
<td>86–177</td>
<td>&lt;500 to 310,000</td>
</tr>
<tr>
<td>Chronic herpes simplex virus infection</td>
<td>2</td>
<td>1</td>
<td>117–207</td>
<td>4300–653,000</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>1</td>
<td>0</td>
<td>105</td>
<td>26,000</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>1</td>
<td>0</td>
<td>33</td>
<td>53,000</td>
</tr>
<tr>
<td>Disseminated histoplasmosis</td>
<td>1</td>
<td>0</td>
<td>19</td>
<td>644,000</td>
</tr>
<tr>
<td>Progressive multifocal leukoencephalopathy</td>
<td>1</td>
<td>0</td>
<td>177</td>
<td>&lt;500</td>
</tr>
<tr>
<td>Total no. of subjects</td>
<td>33</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases per 100 person-years (95% CI)</td>
<td>1.75 (1.21–2.46)</td>
<td>0.73 (0.41–1.25)</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
follow-up HIV RNA load: consistently <500 copies/mL, intermittently >500 copies/mL but consistently <5000 copies/mL, intermittently >5000 copies/mL, and consistently >5000 copies/mL. A separate GEE model was used to estimate trends over time for each of the 4 subgroups. In addition, a unified GEE model was also fit to compare the 4 groups, assuming a common intercept but allowing group by time interaction terms in the model.

GEE models were also used to explore possible factors that might be associated with long-term CD4+ cell counts, including the same covariates considered to be predictors for the risk of AIDS or death, with the exception of baseline CD4+ cell count. In all GEE models of CD4+ cell count trends, an exchangeable correlation matrix was assumed for successive CD4+ cell counts for a subject. Factors with a $P$ value of $\leq .10$ were kept in the final model.

RESULTS

Of the 643 subjects enrolled in the original MAC prophylaxis discontinuation study, CD4+ cell counts and HIV RNA loads for 612 were measured at baseline and at least once during the follow-up period. Of the 31 subjects not included in this analysis, the majority (23 subjects) did not have a baseline specimen submitted for HIV RNA analysis, 1 subject had no baseline assay results because of a lab processing error, and the other 7 subjects were lost to follow-up. At study entry, the median age of the subjects was 40 years, 87% were male, the median CD4+ cell count was 226 cells/mm$^3$, and 63% had an HIV RNA load of <500 copies/mL. Subjects had received previous combination HAART for a median of 43 weeks; the median follow-up duration since entering the MAC prophylaxis discontinuation trial was 184 weeks (range, 8–216 weeks). Details of the baseline characteristics of these subjects have been reported elsewhere [12].

Before enrolling in the MAC prophylaxis discontinuation study, nearly 62% of subjects (396 of 643) had at least 1 AIDS-defining event; almost all participants were receiving some form of PCP prophylaxis. Although only 67%–76% of the subjects were receiving MAC prophylaxis, there was no statistical difference between those who did and those who did not develop an opportunistic infection.

Thirty-three subjects developed at least 1 AIDS-defining opportunistic complication during the 1882 person-years of follow-up, for an overall rate of 1.75 events per 100 person-years (exact 95% CI, 1.21–2.46) (table 1). Of these 33 subjects, 12 had no previous AIDS-defining illness; common AIDS diagnoses among the remaining 21 subjects included PCP, Kaposi sarcoma, esophageal candidiasis, and HIV wasting. There was no difference in the rate of developing an AIDS event or mortality between subjects with and those without a previous AIDS-
defining illness. Among the 33 subjects, 7 experienced >1 type of AIDS-defining event. There was a marginally significant decrease in the rate of opportunistic complications during 1997–2002 ($P = .083$). Of note, >40% of subjects (14 of 33) developed their first opportunistic complication concomitant with a CD4+ cell count >200 cells/mm$^3$. Among these were 4 cases of probable PCP (i.e., pulmonary symptoms that responded to PCP-active therapy but were not confirmed histologically), 3 cases of acute cryptosporidiosis, 2 cases of CMV retinitis (both occurring within 8 months after study entry), 2 cases of clinically diagnosed Candida esophagitis (1 in the context of a transient increase in viral load to 46,000 copies/mL), and 1 case each of chronic herpes simplex virus infection and HIV wasting. In all of these instances, HIV RNA levels were <25,000 copies/mL. There were 2 cases of newly documented, localized MAC osteomyelitis, one occurring at week 20 of the study (concomitant with a CD4+ cell count of 125 cells/mm$^3$) and the other at week 32 (concomitant with a CD4+ cell count of 306 cells/mm$^3$); in both instances, the HIV RNA load was undetectable [11]. Univariate and multivariate Cox proportional hazards models revealed that, among the covariates described in Patients and Methods, only detectable HIV RNA load at baseline showed a significant association with the risk of developing an AIDS-defining event (hazard ratio [HR], 3.88; 95% CI, 1.87–8.06). The relationship between baseline viral load and time to development of a new AIDS-defining event is clearly depicted in figure 1.

A total of 31 subjects died during 1934 person-years of follow-up, for an overall rate of 1.60 deaths per 100 person-years (exact 95% CI, 1.09–2.28). Of the 31 deaths, only 5 were directly related to HIV infection. Other common causes of death recorded were cardiovascular condition (4 cases), liver failure (4 cases, 1 each in a hepatitis B virus–coinfected patient and a hepatitis C virus–coinfected patient), and sepsis (4 cases); causes in 4 cases were unknown. There was no change in the mortality rate during 1997–2002 ($P = .565$). Cox proportional hazards models showed that detectable HIV RNA load (HR, 2.39; 95% CI, 1.38–4.15; $P = .002$) and Karnofsky score of ≤80 (HR, 2.47; 95% CI, 1.37–4.46) were the only factors, among those considered, that were significantly associated with AIDS-free survival.

Changes in mean CD4+ cell counts over time are shown in figure 2. For all subjects, CD4+ cell counts increased by an estimated 5.9 cells/mm$^3$ every 8 weeks. The degree of increase in the CD4+ cell count was associated with the baseline HIV RNA load (<500 vs. ≥500 copies/mL), with greater increases, on average, occurring in those subjects who entered the study

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**Figure 3.** Predicted CD4+ cell counts for 4 HIV RNA detectability groups.

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**Table 2.** Effects of baseline covariates and follow-up duration on absolute CD4+ cell counts.

<table>
<thead>
<tr>
<th>Baseline covariate</th>
<th>Estimated mean change in CD4+ cell count, cells/mm$^3$ (95% confidence limit)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week on study (every 8 weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable HIV RNA load</td>
<td>+7.2 (+6.4 to +8.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Detectable HIV RNA load</td>
<td>+3.4 (+2.0 to +4.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age (every 10 years)</td>
<td>−29.0 (−42.0 to −16.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>+49.0 (+11.9 to +86.2)</td>
<td>.010</td>
</tr>
<tr>
<td>Previous protease inhibitor use for &gt;30 days</td>
<td>+64.2 (+1.4 to +127.1)</td>
<td>.045</td>
</tr>
<tr>
<td>Karnofsky score ≤80</td>
<td>−37.6 (−67.9 to −7.2)</td>
<td>.015</td>
</tr>
<tr>
<td>Less adherent to therapy</td>
<td>−24.9 (−52.6 to +2.8)</td>
<td>.078</td>
</tr>
</tbody>
</table>

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with an undetectable HIV RNA load (an estimated increase of 7.2 cells/mm³ in the CD4⁺ cell count every 8 weeks for subjects with an undetectable HIV RNA load vs. an increase of 3.4 cells/ mm³ for those with a detectable HIV RNA load). Subsequent measurements of virologic suppression (based on HIV RNA load) were also associated with predicted responses in CD4⁺ cell count (figure 3). Subjects with relatively controlled viral replication (i.e., an HIV RNA load consistently <5000 copies/mL) had continuing and sustained increases in CD4⁺ cell counts of ~8 cells/mm³ every 8 weeks, compared with those with an HIV RNA load consistently >5000 copies/mL who had smaller (i.e., ~4 CD4⁺ cells/mm³ every 8 weeks) but gradual decreases in CD4⁺ cell counts. The 95% confidence limits indicated that the trend of changes in the CD4⁺ cell count differed significantly for these 2 groups. There was no obvious difference, however, in the rate of increase in the CD4⁺ cell count between subjects who maintained an HIV RNA load of <500 copies/mL and those who maintained an HIV RNA load of 500–5000 copies/mL.

Table 2 summarizes the association of baseline covariates with trends in absolute CD4⁺ cell counts. When other covariates were held constant, the mean CD4⁺ cell count was 49 cells/mm³ higher among female subjects, compared with male subjects; was 64 cells/mm³ higher among patients who had previously used protease inhibitors for >30 days before entry into the prophylaxis discontinuation study, compared with those who had not; and increased ~7 cells/mm³ every 8 weeks among patients with undetectable baseline HIV RNA loads. Conversely, mean CD4⁺ cell counts decreased by 29 cells/mm³ for every 10-year increase in age, by 38 cells/mm³ among patients with more debilitation (Karnofsky score of ≤80), and by 25 cells/mm³ among patients with less adherence to antiretroviral therapy.

**DISCUSSION**

There were remarkably low numbers of AIDS-defining illnesses over a prolonged follow-up period (1934 person-years) in this study of HIV-infected individuals who had previously been severely immunosuppressed but who had responded to HAART. In the majority of cases, opportunistic illnesses occurred in the context of decreases in CD4⁺ cell counts and suboptimal control of virologic replication. In those cases in which the CD4⁺ cell counts were uncharacteristically high, diagnoses were more often presumptive or clinical presentations were atypical. These observations are in dramatic contrast to the historically high risk of life-threatening opportunistic illnesses and generally poor prognosis for untreated HIV-infected individuals who have markedly diminished CD4⁺ cell counts [28–32]. Even among patients treated with triple-drug therapy, baseline CD4⁺ cell counts <200 cells/mm³ have been associated with a greater risk of disease progression to AIDS and to death, compared with those with higher CD4⁺ cell counts [4]. An interesting observation in this study was that a majority of subjects—even those who did not achieve maximal HIV RNA suppression—continued to show increases in CD4⁺ cell counts for years after starting HAART. Because these subjects had already been receiving potent antiretroviral therapy for a mean duration of >3 years before study entry and the duration of follow-up has been equally long, this observation suggests that immune recovery may continue for >5 years after treatment with potent combination antiretroviral therapy. A similar relationship between CD4⁺ cell response and virological control has been shown in patients who were studied for a shorter period of time and who were not as profoundly immunosuppressed [33]. Likewise, immunologic stability has also been noted in patients with virologic failure, and this relationship has been partially attributed to the decreased virulence of drug-resistant viruses [34].

The initial CD4⁺ cell response after starting HAART predominantly involves memory CD4⁺ cells (CD45RO⁺), followed by a less-pronounced but steady increase in naïve CD4⁺ cells (CD45RA⁺) [35]. Decreases in activated CD4 and CD8 surface markers occur simultaneously and seem to be correlated with viral suppression [36]. Discordant responses among patients, such as increases in the CD4⁺ cell count despite the lack of suppression of viral replication, usually are differentiated by higher numbers of memory CD4⁺ cells and activated (CD38⁺) CD8 cells, as well as by a lower percentage of IL-2–producing CD4⁺ cells [37]. Additionally, a number of studies suggest that, despite suppression of viral replication, functional recovery (as defined by current assays) may be incomplete [23–26]. Although it has been shown that plasma viral load is predictive of the rate of decrease in CD4⁺ lymphocyte counts and progression to AIDS and death [38], it is likely that the relationship between viral suppression and CD4⁺ cell count is dynamic.

Even without maximal virological suppression, some degree of immune recovery occurs following HAART. Because the GEE models used in this analysis should be considered to be exploratory, confirmation warrants further studies in the context of dynamic CD4⁺ cell counts and HIV RNA responses. Opportunistic illnesses and/or complications (including death) are relatively infrequent but can occur at any time, even for patients who maintained increases in the CD4⁺ cell count. Physicians and other professionals who provide AIDS-related care must be aware of atypical presentations or altered natural histories of classic opportunistic infections. There are likely a number of factors that affect immune recovery. The long-term impact on morbidity and mortality can only be assessed by continued monitoring of the durability of immune recovery in unique cohorts of patients, one of which was presented here.

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