Long-Term Outcomes among Antiretroviral-Naive Human Immunodeficiency Virus–Infected Patients with Small Increases in CD4⁺ Cell Counts after Successful Virologic Suppression

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To evaluate the frequency and predictive factors of discordant immune response, we performed a prospective cohort study of 288 antiretroviral-naive human immunodeficiency virus (HIV)–infected patients who initiated highly active antiretroviral therapy (HAART) and maintained complete virus suppression for ≥ 24 months. The median CD4⁺ cell count was 186 × 10⁶ cells/L, and the median HIV RNA level was 5 log₁₀ copies/mL. After 24 months of therapy, 42 (16.5%) of 255 patients had a median CD4⁺ cell count increase of < 100 × 10⁶ cells/L. By logistic regression analysis, previous injection drug use was associated with a CD4⁺ cell count increase of < 100 × 10⁶ cells/L (risk ratio [RR], 2.326; 95% confidence interval [CI], 1.077–5.023; \( P < .032 \)); inclusion of a protease inhibitor (PI) in the HAART regimen reduced the risk of poor immunologic recovery (RR, 0.160; 95% CI, 0.061–0.417; \( P < .001 \)). Failure of the CD4⁺ cell count to increase was relatively common among antiretroviral-naive patients in the year after the initiation of HAART and the achievement of complete virus suppression. PI-containing regimens provided better immunologic response.

Current potent therapy against HIV-1 is associated with immunologic and clinical improvement [1–4]. Quantitative CD4⁺ cell reconstitution is heterogeneous. In some HIV-infected patients, combination therapy with antiretrovirals does not lead to a rapid increase in CD4⁺ cell counts even though it results in well controlled virus replication. Little is known about the factors involved in the pathogenesis of disease and the long-term clinical outcomes for HIV-infected individuals who receive HAART and have “discordant immune responses” (i.e., those whose CD4⁺ cell counts do not increase despite marked reductions in virus load).

Although randomized controlled trials are the “gold standard” in biomedical research, the data from such trials do not accurately reflect clinical reality, and data from daily clinical assessment of patients are necessary for better treatment of HIV-1–infected people. The aim of this study is to describe the immunologic and clinical outcomes for a group of antiretroviral-naive HIV-infected patients who began receiving HAART and had discordant immune responses despite successful reduction of HIV-1 RNA levels.

**PATIENTS AND METHODS**

**Study participants.** This study was performed in the HIV Care Unit at the Hospital Ramón y Cajal in Madrid, Spain. This unit provides primary and specialized care to HIV-infected adults in an urban area with a high prevalence of HIV infection. All antiretroviral-
naive patients who initiated HAART after March 1996 were included in a prospective clinical cohort study that had continuous enrollment and standardized collection of data. Information collected at registration included age, sex, route of acquisition of HIV-1 infection, baseline virus load and CD4+ cell count, results of serologic tests for hepatitis C virus (HCV) and hepatitis B virus (HBV), HIV/AIDS stage, and time since the onset of the first AIDS-defining illness. CD4+ cell counts and HIV RNA levels were determined routinely at follow-up visits that occurred every 3 months at 1 of 4 outpatient clinics. Information about compliance with antiretroviral treatment, side effects, and opportunistic infections was specifically collected.

For the purpose of this study, we selected patients who had ≥24 months of follow-up and maintained complete virus suppression throughout follow-up. The database used for the analysis included information recorded until December 2000. The work was approved by the institutional review board of our institution.

Laboratory tests. CD4+ cell counts were assessed by flow cytometry. Virus loads were measured in plasma samples, initially with the use of a commercial quantitative PCR technique (Amplicor HIV; Roche Diagnostic Systems). Since September 1998, HIV-1 RNA level determination has been performed using an ultrasensitive branched DNA assay (Chiron Diagnostics) that has a lower limit of detection of 50 RNA copies/mL.

Definitions. A “long-term immunologic response” to treatment was defined as a sustained CD4+ cell count increase of >100 × 10^6 cells/L, as measured from baseline, during 24 months of follow-up. All patients who did not meet this criterion were considered to have a “discordant immune response.” Adherence to therapy was quantified as the number of doses reported to have been taken. Adverse events were graded according to the toxicity rating scale developed by the World Health Organization [5]. Patients who experienced clinically significant drug-associated toxicity were treated according to the discretion of the clinicians, who decided whether to continue or to interrupt the course of medication.

Statistical analysis. Descriptive statistics and analytical methods, both parametric and nonparametric, were used to analyze the data. Continuous variables were expressed as medians and ranges. Categorical variables were expressed as the number and percentage of patients in each group. The number of patients with an HIV RNA level of <50 copies/mL—specifically, the number of such patients noted after 12 and after 24 months of therapy—was used as the primary measure of antiretroviral response during the study period. The association between immune discordance and baseline variables was compared using the Mann-Whitney U test or the χ² test or, when necessary, Fisher’s exact test. A stepwise multivariable logistic regression model, which included variables significantly associated with discordant immune response in the univariate analysis, was used to assess the independent effect of each variable on a discordant immune response. Risk ratios (RR) and their 95% CIs were computed. We used Cox proportional hazards models to determine factors associated with the CD4+ cell response. All P values were 2-tailed, and P < .05 was considered statistically significant.

RESULTS

Study patients. From April 1996 through December 2000, a total of 501 antiretroviral-naive HIV-seropositive patients who initiated HAART (regimens containing either a protease inhibitor [PI] or nonnucleoside reverse-transcriptase inhibitors [NNRTIs]) were recruited in the Clinical Cohort Study at the Hospital Ramón y Cajal. No exclusion criteria related to CD4+ cell count or plasma HIV RNA level were applied. We selected a subgroup of 288 HIV-infected individuals who maintained complete virus suppression for ≥2 years and for whom a baseline CD4+ cell count was available. The patient population included 214 men and 74 women, who were predominantly former injection drug users (61%). At baseline, these patients had moderately advanced HIV infection. We observed a well-balanced distribution of patients who had received azidothymidine-based regimens (55.5%) and patients who had received stavudine-based regimens (45.5%). Because of the nature of the study and the availability of new drugs, more patients received PIs (91%) than received NNRTIs (9%). The most frequently used PI was indinavir (in 185 [64%] of 288 patients), and the most frequently used NNRTI was nevirapine (in 19 [76%] of 25 patients who received NNRTIs). The main demographic and clinical characteristics of the study population are summarized in table 1.

CD4+ cell response to HAART: risk factors for a poor immunologic response. Overall, the median increases in the CD4+ cell count were as follows: 178 × 10^6 cells/L at month 12, 260 × 10^6 cells/L at month 24, and 321 × 10^6 cells/L at month 36. A total of 76 (26.4%) of 288 patients had a CD4+ cell count increase of <100 × 10^6 cells/L at 12 months (figure 1). After 24 months of follow-up, 42 (16.5%) of 255 patients were considered to have a discordant immune response, whereas most patients had adequate immunologic recovery (figure 1). It was not uncommon for a patient to have a discordant immune response at 1 year of follow-up and to have a good immunologic response subsequently (42 patients). In contrast, for a small number of patients (14), immune response became discordant between month 12 and month 24.

As determined by univariate analysis, the following risk factors were associated with a discordant immune response: lower virus load at baseline (4.66 log_{10} RNA copies/mL, compared with 5.02 log_{10} RNA copies/mL for patients with a good immunologic response; P = .002), higher CD4+ cell count at base-
Table 1. Baseline demographic and clinical characteristics of 288 HIV-infected patients who had complete virus suppression while receiving HAART and completed at least 2 years of follow-up.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years (range)</td>
<td>36 (19–68)</td>
</tr>
<tr>
<td>Male sex</td>
<td>74</td>
</tr>
<tr>
<td>Risk practice for HIV infection</td>
<td></td>
</tr>
<tr>
<td>Injection drug use</td>
<td>61</td>
</tr>
<tr>
<td>Unsafe sex</td>
<td>33</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
<tr>
<td>Previous diagnosis of AIDS</td>
<td>29</td>
</tr>
<tr>
<td>Coinfection</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>60</td>
</tr>
<tr>
<td>HBV</td>
<td>2</td>
</tr>
<tr>
<td>HCV and HBV</td>
<td>5</td>
</tr>
<tr>
<td>CD4⁺ cell count</td>
<td></td>
</tr>
<tr>
<td>&lt;200 × 10⁶ cells/L at baseline</td>
<td>157 (54)</td>
</tr>
<tr>
<td>Median cells × 10⁶/L (range)</td>
<td>186 (2–1113)</td>
</tr>
<tr>
<td>Median HIV RNA level, log₁₀ copies/mL</td>
<td>5 (2.3–6.5)</td>
</tr>
<tr>
<td>Initial combination therapy</td>
<td></td>
</tr>
<tr>
<td>PI-based regimen</td>
<td>91</td>
</tr>
<tr>
<td>NNRTI-based regimen</td>
<td>9</td>
</tr>
</tbody>
</table>

**NOTE.** Data are % of patients or no. (%) of patients, unless otherwise indicated. HCV, hepatitis C virus; HBV, hepatitis B virus; PI, protease inhibitor; NNRTI, nonnucleoside reverse-transcriptase inhibitor.

* As indicated by a test result positive for hepatitis B surface antigen.

Figure 1. Percentage of patients receiving HAART whose CD4⁺ cell counts increased by < 200 × 10⁶ cells/L during follow-up. DIR, discordant immune response.

**DISCUSSION**

Our data provide evidence that a significant proportion of antiretroviral-naïve, moderately immunocompromised HIV-infected patients who begin receiving HAART have a poor immune recovery and exhibit a late immunologic response, despite having complete virus suppression, for at least 24 months.
The delayed or discordant immune reconstitution was not associated with a poor clinical outcome, except for those patients whose CD4+ cell counts remained < 200 x 10^6 cells/L.

In the French Hospital Database on HIV, it is reported that this kind of discordant immune response (defined as a CD4+ cell count increase of < 50 x 10^6 cells/L from the count at baseline) was detected in 17.3% of patients after 6 months of therapy [6]. The rate we found is also much higher than the 9% rate (at 12 months) reported by Piketty et al. [7] in a prospective cohort study of 150 patients who had previously received dual therapy with nucleoside analogues. In our study, after 2 years of follow-up, a discordant immune response was detected in ~1 of every 6 patients who had started receiving HAART (figure 1).

We have identified 2 independent factors associated with a discordant immunologic-virologic response. First, the probability of having a discordant immune response was significantly higher among patients receiving regimens that contained NNRTIs. In agreement with that finding, 2 studies have shown that in patients with sustained virus suppression in plasma, triple therapy that included an HIV-1 PI was more potent for the reduction of the virus burden in lymphoid tissue than was triple therapy that contained nevirapine or nucleoside analogues alone [8, 9]. Another study suggested that PIs may influence the recovery of the CD4+ T cell count through a nonvirologic effect [10]. Also, a systematic review of results from 23 clinical trials reported in the medical literature found a trend toward superior CD4+ cell responses among patients receiving regimens containing PIs [11]. Combination therapy that included PIs proved to result in superior immune recovery, compared with regimens that included NNRTIs, as manifested by a higher CD4+ cell count after a long period of virologic suppression. Even in patients experiencing virologic failure, continuation of PI therapy may contribute to an immunologic recovery and may possibly provide clinical benefit [12]. This "immunologic-virologic disconnect" could be explained by an increase in thymic output (i.e., replenishment of the CD4+ cell pool, as assessed by quantification of T cell receptor excision circles), and it can contribute to the dissociation between CD4+ cell counts and viremia, even in patients for whom antiretroviral therapy fails [13]. Therefore, PIs may induce a sustained CD4+ cell response for reasons independent of their direct antiviral activity.

The second predictive factor for discordant immune response that we identified was previous injection drug use. In Spain, almost two-thirds of HIV-infected patients acquired the infection by the parenteral route. Coinfection with HCV, which shares the same pathways of transmission, is also especially prevalent in our country. Most individuals in the study population were former injection drug users (61%) and were coinfected with both HIV and HCV (65%). In the Swiss cohort study, almost 90% of HCV-positive patients were former or active injection drug users, and the probability of clinical progression was higher among patients who were coinfected with HCV and/or were injection drug users [14]. The injection of heroin and/or cocaine has been considered an important factor implicated in some degrees of immunocompromise. Our study demonstrated that prior injection drug use, but not coinfection with HCV, delayed and impaired the recovery of the CD4+ cell count. Thus, it seems likely that other forms of impairment of the immune system that are not related to the direct effect of HIV-1 could be involved in the recovery of CD4+ cell counts in patients receiving HAART.

It has been reported that the incidences of AIDS-defining events and death were higher in a group of patients who demonstrated no immunologic recovery despite having a good virologic response (21% of patients) than among patients who demonstrated a full immune response (2% of patients) [7]. In another study, the rate of progression to AIDS or death at 24 months was also higher in the group with discordant immune response (9.5% of patients) than in the group with the complete response (4.8% of patients) [6]. In our study, the incidence of clinical events at 24 months was highest among those participants whose CD4+ cell counts remained < 200 x 10^6 cells/L, but we could not find significant differences between the incidence of clinical events among patients whose CD4+ cell counts increased by > 100 x 10^6 cells/L and among patients whose CD4+ cell counts increased by less than that value. Herpes zoster infection was the most common opportunistic infection noted in our series. It has been pointed out that herpes zoster infection is detected in 7%–8% of patients who initiate HAART, typically during weeks 4–16 after the initiation of therapy [15, 16]. The development of this viral complication is significantly associated with a more vigorous CD8+ response [15, 16].

Determination of the factors associated with long-term clinical outcomes early in the course of treatment may allow therapy to be modified for patients who have a greater risk for immunologic treatment failure. Our series provides evidence that, for a significant proportion of patients with a discordant immune response to HAART in the first year of virus suppression, the discordant response resolved during the subsequent year of follow-up (figure 1). Some studies have evaluated the use of IL-2, an immunomodulator agent recently used as therapy for HIV-infection, to improve the immunologic response [17–20, 22]. After receiving only 3 cycles of IL-2 immunotherapy, a small group of patients whose CD4+ cell counts had remained < 200 x 10^6 cells/L after they had received HAART for > 9 months demonstrated a marked increase in CD4+ cell counts [18]. Similarly, a recent report described 15 patients who had discordant immune responses to HAART and CD4+ cell counts that remained < 300 x 10^6 cells/L and who received 3 cycles of IL-2. The IL-2 therapy increased the CD4+ cell counts in 74% of patients and, for some of these patients, immunologic recovery permitted the discontinuation of pro-
phylaxis against *Pneumocystis carinii* pneumonia, because patients maintained CD4\(^+\) cell counts of > 200 \times 10^3\) cells/L [20, 21]. Therefore, IL-2 immunotherapy can accelerate the recovery of CD4\(^+\) cell counts in patients with low CD4\(^+\)-cell response, but the long-term clinical benefit of such therapy remains to be proven [22].

Our study has several limitations. First, it was a non-randomized, observational, clinical cohort study, and, at the time of initiation of PI-based or NNRTI-based therapy, the population was not well-balanced. The selection of PI-based or NNRTI-based therapy depended on the medical criteria and the availability of the treatment regimens; NNRTIs were not available in Spain until the second half of 1998. Second, most patients were severely immunocompromised (median CD4\(^+\) cell count at baseline, 186 \times 10^3\) cells/L), and this fact could have favored the selection of a PI-based regimen. Finally, we cannot exclude the possibility that our results were distorted by residual confounding factors or unmeasured factors.

In summary, failure of the CD4\(^+\) cell count to increase despite successful virologic suppression is relatively frequent among antiretroviral-naïve HIV-infected patients who are moderately severely immunocompromised at the time of initiation of HAART. After a long period of virus suppression and a long follow-up, regimens that included a PI resulted in better immunologic response than did regimens that contained NNRTIs. Prior use of nonprescribed injection drugs was associated with a poorer immunologic response. Further studies are needed to confirm these observations, to assess alternative therapeutic measures, and to clarify their implications for better clinical management of HIV-infected patients.

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**References**


