This study investigated whether immune restoration occurred in 26 human immunodeficiency virus (HIV) type 1–infected children treated first with indinavir for 16 weeks and then with combination antiretroviral therapy for >2 years. Compared with baseline, a significant, although modest, decrease in virus loads (maximum median, −0.86 log₁₀) and increase in the number of CD⁴⁺ lymphocytes, especially naive cells, were observed at several time points after 2 years. A maximum of 7% of treated children achieved undetectable viremia. There was a marked increase in the proliferative response and skin reactivity to recall antigens. However, responses to an HIV antigen remained depressed, and the production of interleukin-12 remained unchanged and abnormally low. The magnitude of virus suppression did not correlate with these measures of functional immune reconstitution. These findings suggest that long-term nonsuppressive antiretroviral therapy can induce limited improvement in immune function in pediatric AIDS patients and that the effect of suppressive treatments should be investigated.

Infection with human immunodeficiency virus (HIV) type 1 results in severe immune dysfunction that affects both T lymphocytes and antigen-presenting cells (APCs) [1]. The advent of protease inhibitors and combination therapy has led to significant improvement of virus suppression, increases in numbers of CD⁴⁺ T cells, and declines in morbidity and mortality [2]. However, there is less information on the effects of such therapy on immune function, especially after long-term treatment [3]. Moreover, few studies have investigated the effect of therapy on APC function [4, 5]. Information available on changes in immune function in treated HIV-infected children is even more limited.

To address some of these issues, a longitudinal study of the effect of treatment on immune reconstitution was conducted in HIV-infected children enrolled in a study of the protease inhibitor indinavir given alone and then in combination with zidovudine and lamivudine [6]. This regimen is now recognized as suboptimal, because the drug therapies were not all started simultaneously and the doses of indinavir were somewhat lower than those currently recommended. Most of these children had severe immune dysfunction and extensive prior antiretroviral therapy. We previously reported that, despite significantly decreased virus loads and increased CD⁴⁺ cell counts at week 28 of therapy, restoration of defective cellular immune responses did not occur at that time [7]. Furthermore, no changes were detected in the cytokine profile, with the exception of decreased soluble tumor necrosis factor (TNF) receptor II levels [7]. In the present study, we extended data from the 6-month report [7] and investigated whether immune function was restored after longer-term treatment of the same children. Here we describe the immunologic and virologic parameters in the patients who remained in the study for 2–3 years of follow-up.

**Patients and Methods**

**Patient population.** From July 1995 through August 1996, 54 HIV-infected children aged 3–19 years were enrolled in a phase 1/2 study of indinavir, which was administered as monotherapy for the first 16 weeks, followed by the addition of zidovudine and lamivudine [6, 8]. Blood samples were obtained before enrollment (week 0) and at weeks 120, 144, and 168 after treatment initiation.

**Immunologic parameters.** Immunologic assays were performed on fresh peripheral blood mononuclear cells (PBMC) [7]. Cytokine production was measured by ELISA on 24-h supernatants from
Staphylococcus aureus Cowan (SAC)–stimulated PBMC (Calbiochem). Lymphocyte proliferation assays were performed in response to phytohemagglutinin (PHA)–M (Life Technologies); a mixture of recall antigens: Candida albicans antigen (Greer Laboratories), influenza virus A, and tetanus toxoid (TT; Connaught Laboratories); and inactivated gp120-depleted HIV-1 antigen (Immune Response). Results were expressed as stimulation indexes (SIs), as follows: [counts per minute (cpm) of stimulated cultures]/[cpm of unstimulated cultures]. Skin tests for C. albicans antigen (1:100) and TT (1:5) were considered to be positive if the induration was ≥5 mm, regardless of erythema. To determine virus load, plasma HIV RNA was quantified by reverse transcriptase–polymerase chain reaction assay (detection limit, 200 copies/mL; Amplicor, Roche).

Lymphocytes and CD4+ T cell subsets. Lymphocytes and lymphocyte subsets were determined by flow cytometry analysis. CD4+ “naive” cells were identified as CD4+/CD45RA+/CD45RO or as CD45RA–CD62L−.

Statistical methods. Changes in parameter values between 2 time points were made by subtracting the baseline value from that at a later time point, except for the virus load, for which the change was log_{10} (virus load at baseline/virus load at a later time point).

We used the Wilcoxon signed-rank test to determine whether the changes from baseline were significant. Changes in skin test reactivity were tested by the McNemar test for paired categorical data. P values were adjusted for multiple comparisons by the Hochberg method. To test for associations between changes in virus load and in other parameters, a Spearman’s rank correlation coefficient was calculated, using the following interpretation of r: strong association, r > 0.7; moderate association, r = 0.5 to ≈0.7; moderate to weak association, r = 0.3 to <0.5; weak association, r < 0.3. P values for the correlation analysis were not adjusted for multiple comparisons.

Results

Baseline characteristics. Median baseline age, CD4+ T cell count, and virus load, respectively, were 8.5 years (range, 3.1–15.3 years), 318 cells/mL (0–1706 cells/mL), and 4.64 log_{10} HIV RNA copies/mL (3.68–5.77 copies/mL) for the 26 children. Of the 26 children, 23 had acquired HIV infection through vertical transmission and 23 had extensive prior exposure to nucleoside reverse-transcriptase inhibitors. In comparison with the 28 children who were enrolled [7] but not followed up, the 26 children in the present study had similar virus loads (P = 0.54, Wilcoxon rank sum test) and higher CD4+ cell counts (P = 0.04) at entry. Of the 28 children, 18 were withdrawn for miscellaneous reasons not related to worsening clinical or immunologic status, 5 for toxic effects, 3 for clinical progression, and 2 for immunologic decline.

Therapy-associated changes. This longitudinal study permitted analysis of changes for individual patients at multiple time points. Median virus loads were decreased at all time points, compared with entry levels (figure 1A). The median virus loads were 3.9, 3.7, and 3.0 log_{10} HIV RNA copies/mL at weeks 120, 144, and 168, respectively. However, only 2 of 26 children had an undetectable virus load at week 120, 2 of 23 at week 144, and one of 16 at week 168. Median CD4+ cell counts were increased at all time points, but these differences reached statistical significance only at week 144, when compared with cell counts at week 0 (figure 1B; median cell counts at weeks 120, 144, and 168, respectively, were 541, 578, and 648 cells/mL). The numbers of naive (CD45RA+) T lymphocytes demonstrated an increasing trend with time, and the difference from baseline was significant at weeks 120 and 144 (figure 1B).

At enrollment, PHA-stimulated proliferation of PBMC from the patients was reduced, compared with HIV-uninfected controls (data not shown), but was not absent, since 55% (11/20) of the patients had an SI >10, compared with 95% (36/38) of the HIV-uninfected donors. After >120 weeks of therapy, the PHA response was not significantly different from that at baseline (figure 1C). Pooled recall antigen responses were almost completely absent at enrollment (only 8% of HIV-infected children had an SI >3, vs. 82% of HIV-uninfected donors). By contrast, as shown in figure 1C, the changes in SI levels from baseline demonstrated an increasing trend with time, and the difference was significant at week 144. However, enhanced response to recall antigen was not accompanied by a significant increase in response to HIV antigen (P = .69 and .09 at weeks 144 and 168, respectively; figure 1C). Skin test responsiveness to C. albicans antigen substantially increased, whereas the number of children with reactivity to TT did not increase and remained very low (table 1). Because CD4+ and CD4+CD45RA+ counts had increased in most patients by week 120, we analyzed whether the changes in skin test reactivity correlated with those increases. The children who became responsive to C. albicans antigen had significantly higher increases in CD4+ cells (P = .02, Wilcoxon rank sum test) and had a trend toward an increase in CD45RA− cells, compared with children who remained nonresponsive (P = .07). By contrast, improved reactivity to TT was not correlated with changes in CD4+ or CD45RA− T cell counts (both P >.5).

Because cytokine production from APCs from HIV-infected patients is dysfunctional [1], we analyzed the longitudinal changes in the cytokine profile of SAC-stimulated PBMC. As shown in figure 1D, no noteworthy change in the production of interleukin (IL)–12, IL–10, or TNF-α was observed. In particular, IL–12 production remained severely impaired.

Relation between immunologic parameters and control of viremia. Because viral replication was not completely controlled by treatment in the majority of children, we comparatively analyzed the changes in virus load between weeks 0 and 120 and the differences in cytokine production and proliferation during the same period. All associations between changes in virus load and differences in immunologic parameters were weak (r <0.35 and P >.2 for all differences).

Discussion

The present study assessed whether long-term antiretroviral treatment of HIV-infected children would significantly improve
cellular immune function. It is now recognized that the regimen used was suboptimal because of the initial indinavir monotherapy and because the indinavir doses (250–500 mg/m² every 8 h initially, followed by 350 mg/m² every 8 h) were lower than the currently recommended pediatric dose (500 mg/m² every 8 h) [6, 8]. This group of patients experienced a significant, although modest, decrease in virus load at all time points, although viral replication was generally not suppressed below the limit of detection. Several reports suggest that some HIV-infected adults and children can have significant increases in CD4+ lymphocyte counts despite incomplete virus suppression [9, 10]. We observed this in some patients during the initial 96

Figure 1. Box plots showing therapy-associated changes (differences between value at a time point vs. baseline value) in immunologic and virologic parameters in 26 human immunodeficiency virus (HIV)–infected children treated for >2 years with combination antiretroviral therapy. A, Changes in log virus loads. B, Changes in absolute numbers of CD4+ and CD4+CD45RA+ T cells. C, Changes in proliferative responses to phytohemagglutinin (PHA), recall antigen (Ag), and HIV antigen (results expressed as changes in stimulation indexes [SIs]). D, Changes in production of cytokines in 24-h supernatants of Staphylococcus aureus Cowan–stimulated cultures. Horizontal bars within boxes correspond to the median; box limits correspond to the 25th and 75th percentiles; vertical lines extend to the 10th and 90th percentiles; values above the 90th or below the 10th percentile are plotted as points. *Significant difference between baseline and the time point considered (P < .05, Wilcoxon signed-rank test, after adjustment). Parenthetical nos. below box plots are the no. of paired samples considered in the analysis (nos. vary because the yield of peripheral blood mononuclear cells recovered was not sufficient to perform all assays at each time point). IL, interleukin; PCR, polymerase chain reaction; TNF, tumor necrosis factor.
weeks of the current trial [8]. During the same period, the numbers of CD4+ T cells increased, in particular those with a naive phenotype, which might reflect the greater thymic function in these younger patients.

An important unanswered question when we initiated this study was whether increases in numbers of CD4+ T lymphocytes resulting from antiviral treatment were associated with restoration of immune function. After >2 years of therapy, we observed substantial increases in the proliferative response to pooled recall antigen and in skin reactivity to C. albicans antigen. These findings were in contrast with our earlier results in the same patient population after a shorter (6-month) treatment period [7]. However, delayed-type hypersensitivity responses to TT remained depressed. Our data support the hypotheses that reexposure to antigen is critical for reconstitution of immune responses in HIV-infected patients [11] and that this could be achieved in children even in the presence of a failing regimen. At the same time, proliferative responses to an HIV antigen did not increase, in agreement with 2 recent reports [11, 12], although the same HIV antigen induced strong T cell proliferation after therapeutic vaccination of HIV-infected adults [13].

Several mechanisms, including selective killing of HIV-specific T cells by infection with HIV or anergy of these cells induced by inhibitory viral factors, could be responsible for the lack of improvement in HIV-specific immune responses. The children in this study achieved only partial virus suppression, and it remains to be seen whether prolonged complete virus suppression will induce greater improvement in immune function. We hypothesize, however, that immunization with HIV or infrequently encountered recall antigen will be needed to induce or reconstitute broad-spectrum immune responses in HIV-infected patients. However, responses to such vaccinations are likely to depend on the degree of HIV suppression. In this regard, therapeutic vaccination of HIV-infected adults with an HIV immunogen led to strong responses to HIV antigen [13], whereas the same immunogen induced only modest responses in HIV-infected children with relatively high virus loads [14].

No significant changes in the production of monocyte-derived cytokines occurred, and IL-12 production, in particular, remained very low. The profound deficit of APC function in HIV-infected children might contribute to their increased susceptibility to infection and poor response to standard immunizations. Asymptomatic adults treated early during the course of infection exhibited conserved IL-12 production [5]. By contrast, production of heterodimeric IL-12 was undetectable after antiretroviral treatment of HIV-infected adults, despite control of viral replication [4]. In addition, we previously described a profound and early suppression of IL-12 production in perinatally infected infants [15]. Most children in the present study were perinatally infected and had advanced HIV disease. Therefore, different factors, including age at the time of infection and the time at which treatment is instituted, influence the effect of therapy on APC function. These results also underscore the necessity of assessing whether earlier and more efficient antiretroviral treatments that lead to complete virus suppression would induce the restoration and maintenance of IL-12 production in HIV-infected children.

Our study provided no clear indication that the magnitude of virologic suppression correlated with measures of functional immune reconstitution. Although the relatively few patients in the study might not have allowed us to document such a correlation, this result is consistent with findings obtained in HIV-infected adults receiving long-term therapy [11]. The persistence of APC dysfunction is an important factor to consider, because it might warrant the use of additional immune modulators. Thus, the development of immune-based therapeutic interventions in conjunction with potent antiretroviral therapy may be essential to achieve immune reconstitution in HIV-infected patients, especially in those with advanced disease and incomplete virus suppression.

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