Longitudinal Analysis of Lymphocyte Ratios and HIV-1 Intracellular DNA Levels in Children

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The associations between human immunodeficiency virus type 1 (HIV-1) intracellular DNA and immunological markers were analyzed longitudinally for children with sustained, undetectable RNA levels while receiving highly active antiretroviral therapy (HAART) for >2 years. When DNA levels reached a plateau at week 104 of therapy, in contrast to findings for adults, there was no correlation between the CD4+ : CD8+ ratio and DNA levels (ρ = −0.02; P = .95), and naive CD4+ CD45RA+ lymphocytes predominated. These data suggest that the increased proportion of naive lymphocytes found in children are less susceptible to HIV-1 infection than are the memory lymphocytes that dominate immune reconstitution in adults.

The availability of antiretrovirals that inhibit HIV-1 at multiple sites (i.e., highly active antiretroviral therapy [HAART]) has led to a significant decrease in death and delayed progression of disease among persons infected with HIV-1 [1]. In association with a decrease in plasma HIV-1 RNA levels, there is a gradual increase in CD4+ lymphocyte counts in children [2, 3]. During long-term use of HAART, individuals usually experience a gradual decrease in CD8+ lymphocyte counts [4], with a resulting increase in the CD4+:CD8+ lymphocyte ratio.

Although current antiretroviral therapy has resulted in sustained virologic suppression for many children infected with HIV-1 [2, 3, 5], variability in CD4+ lymphocyte recovery has been observed. The reasons for variable immune restoration are unknown. It is likely, however, that ongoing low-level viral replication is an important determinant, despite the absence of detectable RNA in plasma. Chun et al. [6] reported that CD4+ : CD8+ ratios and HIV-1 DNA levels were inversely correlated in HIV-1–infected adults who were receiving HAART and whose RNA levels had been undetectable for >2.5 years. Incomplete inhibition of residual ongoing viral replication may be responsible for abnormal CD4+ : CD8+ ratios in HIV-1–infected adults who carry higher levels of HIV-1 DNA, although RNA levels remain undetectable in plasma.

During receipt of HAART, children experience different immune reconstitution than do adults [7]. Although, in children, repopulation of lymphocytes involves predominantly naive CD4+ lymphocytes (CD45RA+), repopulation of lymphocytes in adults involves predominantly memory CD4+ lymphocytes (CD45RO+). To determine whether an association of CD4+ : CD8+ ratios with HIV-1 DNA levels is observed in children receiving HAART, we performed a longitudinal analysis to examine the correlation of HIV-1 DNA levels with immunological markers, including CD4+ and CD8+ lymphocyte counts and CD4+:CD8+ ratios, in HIV-1–infected children with sustained, undetectable RNA levels who had received potent antiretroviral therapy for >2 years.

Materials and methods. Study subjects participated in Pediatric AIDS Clinical Trial Group (PACTG) 382, a study designed to evaluate the pharmacokinetics, tolerance, and potential efficacy of efavirenz, nelfinavir, and ≥1 nucleoside analogue reverse-transcriptase inhibitors (NRTIs) in children. Although patients who were enrolled in the present study were protease inhibitor and nonnucleoside reverse-transcriptase inhibitor naive, they could have received NRTIs previously. The combination therapy was well tolerated and had a potent and sustained antiviral effect in HIV-1–infected children [3].

Selection of the 31 children (median age, 5.6 years; range, 3.2–16.8 years) who were included in the present study was based on HIV-1 RNA levels in the children being persistently undetectable for >2 years after initiation of HAART. These children satisfied the following criteria: (1) they had received study treatment for >2 years, (2) they had sustained HIV-1 RNA levels of <50 copies/mL, and (3) they had no more than

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Figure 1. Median levels of absolute CD4+ lymphocyte counts, CD8+ lymphocyte counts, CD4+:CD8+ ratios, and HIV-1 intracellular DNA during highly active antiretroviral therapy (HAART). Median CD4+ lymphocyte counts increased throughout treatment, with a concomitant increase in the CD4+:CD8+ ratios occurring. HIV-1 DNA levels, expressed as the number of copies/10^6 CD4+ lymphocytes, decreased gradually throughout HAART and reached a plateau between weeks 80 and 104 of treatment. n, Nos. of patients available for the analysis of absolute CD4+ lymphocyte counts, CD8+ lymphocyte counts, and CD4+:CD8+ ratios.

2 detectable HIV-1 RNA measurements of >50 copies/mL (and <1000 RNA copies/mL) after a level of <50 copies/mL had been reached. Detailed data on patient demographics have been reported elsewhere [8]. All patients received NRTIs before entry into the study: 12 patients (39%) received 1 NRTI, and 19 patients (61%) received 2 NRTIs.

Informed consent was obtained from the study participants. The study followed the human experimentation guidelines of the US Department of Health and Human Services and the review board of the University of California, San Diego.

HIV-1 DNA levels were quantified using the Amplicor monitor HIV-1 DNA assay (Roche Molecular Systems) [9], and they were retrospectively quantified using stored peripheral blood mononuclear cells (PBMCs) at baseline and at weeks 2, 4, 8, 20, 48, 80, and 104. The HIV-1 DNA levels were calculated and were expressed as the number of HIV-1 DNA copies/10^6 CD4+ lymphocytes, by use of available CD4+ lymphocyte counts from the same day that PBMCs were available.

The HIV-1 RNA level was measured using a commercially available polymerase chain reaction (PCR) assay (Amplicor HIV-1 Monitor assay [Roche Molecular Systems]). Plasma samples with <400 HIV-1 RNA copies/mL were retested using the Ultrasensitive HIV-1 Monitor assay (version 1.0; Roche Molecular Systems), which has a quantitation limit of 50 HIV-1 RNA copies/mL. The values of the ultrasensitive assay were used for data analysis when samples were retested.

The percentages and absolute numbers of CD4+ and CD8+ lymphocytes were determined in PACTG-certified laboratories, according to the PACTG consensus protocol for flow cytometric analysis. CD4+:CD8+ ratios were calculated using the absolute numbers of CD4+ and CD8+ lymphocytes at baseline and at weeks 2, 4, 8, 20, 48, 80, and 104, when CD4+ and CD8+ lymphocyte counts were available from the same day that PBMCs were obtained.

The quantitation of naive lymphocytes and memory lymphocytes was performed at a PACTG immunology core laboratory. CD4+ lymphocytes that expressed CD45RA+ surface antigen (CD4+CD45RA+) were considered to be predominantly naive CD4+ lymphocytes, and CD4+ lymphocytes that expressed CD45RO+ surface antigen were considered to be predominantly memory CD4+ lymphocytes (CD4+CD45RO+). The data for subsets of naive and memory lymphocytes were available for a subset of patients at week 20 (n = 11), week 48 (n = 13), and week 104 (n = 18).
Table 1. Correlation between HIV-1 DNA and immunological markers during highly active antiretroviral therapy.

<table>
<thead>
<tr>
<th>Time</th>
<th>For CD4⁺ lymphocyte counts</th>
<th>For CD8⁺ lymphocyte counts</th>
<th>For CD4⁺:CD8⁺ lymphocyte ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n = 31)</td>
<td>−0.35 (.06)</td>
<td>0.33 (.08)</td>
<td>−0.59 (&lt;.001)</td>
</tr>
<tr>
<td>Week 2 (n = 24)</td>
<td>−0.36 (.09)</td>
<td>0.43 (.04)</td>
<td>−0.66 (&lt;.001)</td>
</tr>
<tr>
<td>Week 4 (n = 26)</td>
<td>−0.26 (.20)</td>
<td>0.27 (.18)</td>
<td>−0.54 (&lt;.005)</td>
</tr>
<tr>
<td>Week 8 (n = 23)</td>
<td>−0.28 (.19)</td>
<td>−0.15 (.49)</td>
<td>−0.29 (.18)</td>
</tr>
<tr>
<td>Week 20 (n = 22)</td>
<td>−0.17 (.44)</td>
<td>0.08 (.75)</td>
<td>−0.29 (.20)</td>
</tr>
<tr>
<td>Week 48 (n = 26)</td>
<td>−0.34 (.12)</td>
<td>0.22 (.32)</td>
<td>−0.46 (.03)</td>
</tr>
<tr>
<td>Week 80 (n = 20)</td>
<td>0.08 (.74)</td>
<td>0.31 (.19)</td>
<td>−0.31 (.19)</td>
</tr>
<tr>
<td>Week 104 (n = 21)</td>
<td>−0.17 (.47)</td>
<td>−0.03 (.89)</td>
<td>−0.02 (.95)</td>
</tr>
</tbody>
</table>

NOTE. Spearman correlation analysis was used for comparisons of HIV-1 DNA and immunological markers.

Nonparametric tests were used to avoid the assumption of normality. Correlations between the CD4⁺:CD8⁺ ratio and the CD4⁺ lymphocyte count, the CD8⁺ lymphocyte count, the number of HIV-1 DNA copies/10⁶ CD4⁺ lymphocytes, and patient age at entry into the study were calculated using Spearman rank-order methods. For evaluation of CD4⁺ lymphocyte counts, CD8⁺ lymphocyte counts, and the CD4⁺:CD8⁺ ratio, the Wilcoxon matched-pairs signed-rank test was used.

Results. All patients had detectable plasma virus levels with median HIV-1 RNA levels of 7175 copies/mL (range, 788–156,417 copies/mL) before receiving HAART. The changes in the median CD4⁺ lymphocyte count, the median CD8⁺ lymphocyte count, the median CD4⁺:CD8⁺ ratios, and the median HIV-1 DNA levels during HAART are shown in figure 1. The gradual increase in CD4⁺ lymphocyte counts, in association with unchanged CD8⁺ lymphocyte counts, led to an increase in CD4⁺:CD8⁺ ratios throughout HAART, from a ratio of 0.83 at baseline (n = 31; range, 0.28–2.44) to a ratio of 1.11 at week 104 (n = 27; range, 0.44–2.93; P = .0001).

The median HIV-1 DNA levels were compared at each study visit during receipt of HAART (figure 1). The HIV-1 DNA levels expressed per 10⁶ CD4⁺ T cells decreased gradually throughout administration of HAART and reached a plateau between weeks 80 and 104. However, HIV-1 DNA remained detectable in all patients through week 104.

To assess whether patient age has an impact on CD4⁺:CD8⁺ ratios, the association between age and CD4⁺:CD8⁺ ratios was evaluated for each patient. There was an inverse correlation between age and CD4⁺:CD8⁺ ratios before initiation of HAART (n = 31; r = −0.45; P = .01). This negative correlation remained (n = 31; r = −0.44 to −0.30; P = .01–.50) between weeks 2 and 8 during the early stage of HAART. The negative correlation was still observed at week 20 (n = 27; r = −0.47; P = .01), week 48 (n = 31; r = −0.34; P = .06), week 80 (n = 31; r = −0.26; P = .15), and week 104 (n = 27; r = −0.37; P = .06) during HAART.

Before initiation of HAART, the association of various immune parameters with the quantity of HIV-1 DNA in 10⁶ CD4⁺ lymphocytes was evaluated (table 1). There was a marginal inverse correlation between HIV-1 DNA levels and CD4⁺ lymphocyte counts (r = −0.35; P = .06), and there was a marginal positive correlation between HIV-1 DNA levels and CD8⁺ lymphocyte counts (r = 0.33; P = .08). However, when these 2 values were combined and were calculated as CD4⁺:CD8⁺ ratios, there was a strong inverse correlation between CD4⁺:CD8⁺ ratios and HIV-1 DNA levels (r = −0.59; P < .001).

Similarly, during the early stage of administration of HAART, a strong, significant inverse correlation between CD4⁺:CD8⁺ ratios and HIV-1 DNA persisted at week 2 and week 4, but it was no longer statistically significant at week 8, when 16 (52%) of 31 of patients had HIV-1 RNA levels of <50 copies/mL (table 1).

The same immunologic markers and HIV-1 DNA were evaluated at week 104, when median HIV-1 DNA levels reached a plateau (figure 2). No significant correlations were observed between the number of HIV-1 DNA copies/10⁶ CD4⁺ lymphocyte counts and (1) the CD4⁺ lymphocyte count (r = −0.17; P = .47) (figure 2A) and (2) the CD8⁺ lymphocyte count (r = −0.03; P = .89) (figure 2B) at week 104. Furthermore, no correlation was observed between HIV-1 DNA levels and CD4⁺:CD8⁺ ratios at week 104 (r = −0.02; P = .95) (figure 2C).

Naive CD4⁺ lymphocytes continuously were the dominant lymphocyte subset in all children experiencing sustained virologic suppression. The median absolute numbers of naive CD4⁺ lymphocytes and memory CD4⁺ lymphocytes during receipt of HAART were 503 lymphocytes/µL and 232 lymphocytes/µL, respectively, at week 20 (n = 11), and 525 lymphocytes/µL and 257 lymphocytes/µL, respectively, at week 48 (n = 13). At week 104 (n = 18), naive CD4⁺ lymphocytes were the dominant CD4⁺ lymphocyte population, constituting 56% of CD4⁺ lymphocytes (median, 559 lymphocytes/µL; range, 146–1428 lymphocytes/µL), compared with CD45RO⁺, which comprised 33% of CD4⁺ lymphocytes (median, 300 lymphocytes/µL; range, 16–893 lymphocytes/µL).

Discussion. The association of various immunologic markers with HIV-1 DNA levels was evaluated longitudinally in HIV-1–infected children with sustained undetectable HIV-1 RNA levels who received HAART for ≥2 years. There were strong inverse correlations between CD4⁺:CD8⁺ ratios and the number of HIV-1 DNA copies/10⁶ CD4⁺ lymphocytes before and during the early stages of treatment with potent antiretrovirals. A weak correlation between the 2 values persisted...
Figure 2. Association of the no. of HIV-1 intracellular DNA copies/10^6 CD4^+ lymphocytes with immunologic parameters. Associations of HIV-1 DNA copies/10^6 CD4^+ lymphocytes with CD4^+ lymphocyte counts (A), CD8^+ lymphocyte counts (B), and CD4^+ :CD8^+ ratios (C) at week 104 of treatment are shown. Spearman correlation analysis was used for comparisons of HIV-1 DNA and immunological markers.

while HIV-1 DNA levels were decreasing during HAART. However, there was no correlation at week 104, when HIV-1 DNA levels were at a plateau. The findings at the time of prolonged sustained virologic suppression differ from previously published findings for adults who were successfully treated with HAART, which showed a strong inverse correlation between CD4^+ :CD8^+ ratios and the levels of HIV-1 DNA [6].

There are at least 2 possible explanations for why our findings for children differ from those recently reported for adults. First, the different patterns of lymphocyte reconstitution observed in adults and children after successful antiretroviral therapy may alter the quantity of HIV-1 DNA present in the total CD4^+ lymphocyte population. In children, CD45RA^+ naive CD4^+ lymphocytes are the predominant cell population that expands after the initiation of HAART, likely because of the existence of a functional thymus [7]. In adults, however, CD4^+CD45RO^+ lymphocytes are the main cell population that is expanded during successful treatment, followed by CD45RA^+ lymphocytes, which increase more slowly [10]. Similarly, in the present study, CD45RA^+ naive lymphocytes were the dominant CD4^+ lymphocyte subset observed during sustained virologic suppression. The data from the present study indicate that, in children, changes in naive CD4^+ lymphocytes versus memory CD4^+ lymphocytes differed from the changes that would be expected in adults and, thus, could account for the difference between the findings of the present study and the findings of studies of adults.

The hypothesis that the proportion of naive CD4^+ lymphocytes that constitute immune reconstitution in children is a major contributor to the differences observed in the findings of the present study, compared with the findings of studies of adults, is further supported by the differential susceptibility of these cell types to HIV-1 infection [11]. In studies of both adults and children, CD45RO^+ memory lymphocytes are significantly more susceptible to HIV-1 infection than are CD45RA^+ naive lymphocytes [11, 12]. In fact, in one study, the quantity of integrated HIV-1 DNA in CD45RO^+ memory lymphocytes was estimated to be up to 16-fold higher than that in CD45RA^+ naive lymphocytes [12]. A lower expression of CCR5 on the surface of CD45RA^+ naive lymphocytes, compared with CD45RO^+ memory lymphocytes, may also be responsible, in part, for the lower infectability of naive cells by certain strains of HIV-1 [13].

Another possible reason for the disparity in findings observed among children and adults is the impact of age on CD4^+ :CD8^+ ratios in children. In the present study, an inverse correlation between age and CD4^+ :CD8^+ ratios was seen throughout HAART. The CD4^+ :CD8^+ ratios in healthy children are higher than those in adults, and these ratios decrease with advancing age [14, 15]. Before initiation of HAART and during the early stages of HAART (weeks 0–4), the correlation between age and...
HIV-1 DNA levels ($r = -0.30$ to $-0.45; P = .01–.11$) was less statistically significant than the correlation between CD4+:CD8+ ratios and HIV-1 DNA levels ($r = -0.54$ to $-0.66; P < .005$), which suggests that patient age has less of an impact on HIV-1 DNA levels than do CD4+:CD8+ ratios.

In summary, the inability of the CD4+:CD8+ ratio to predict HIV-1 DNA levels in children, compared with adults, is likely the result of differential expansion of the naive CD4+CD45RA+ lymphocyte population noted in children who are less susceptible to HIV-1 infection, compared with the memory CD4+CD45RO+ lymphocyte population noted in adults during immune reconstitution.

References


