CONCISE COMMUNICATION

Effect of HIV on Thymic Function before and after Antiretroviral Therapy in Children


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Studies were undertaken to investigate the role of the thymus in T cell reconstitution in human immunodeficiency virus (HIV)-infected children treated with antiretroviral therapy. Nine pediatric patients who acquired HIV perinatally were treated with multidrug combinations of antiretroviral agents. Plasma virus load and CD4+ and CD8+ T cell subsets were measured, and thymus function was measured by quantifying T cell receptor rearrangement excision circles in peripheral blood. Patients with virus loads remaining >400 RNA copies/mL plasma were classified as virologic nonresponders. Thymus function was initially decreased in all subjects. After antiretrovirus therapy, peripheral CD4+ T cells increased in all subjects. Thymus function was restored in 4 of 5 virologic responders but in only 1 of 4 virologic nonresponders. This suggests that HIV has an adverse effect upon thymic function in pediatric HIV infection. Potent antiretroviral therapy restores thymic function but is affected by the degree to which virus suppression is achieved.

Human immunodeficiency virus (HIV) infection of children and adults leads to a profound decrease in the number and percentage of CD4+ T cells. The mechanisms underlying this depletion are likely to be a composite of peripheral CD4+ T cell depletion by HIV and inadequate replacement of the destroyed T cells [1]. Peripheral T cells can be replaced either by production of new naïve T cells from the thymus or by peripheral expansion of existing naïve and memory T cells within the periphery [2, 3]. The involution of the thymus with age [4] and the resulting inability to replace lost T cells may be partially responsible for the more rapid disease course of HIV infection in older adults [5]. In contrast, the thymus is highly active in children and should aid in maintaining CD4+ T cell numbers during pediatric HIV infection. This fact may help explain why the higher virus loads that are generally observed in children (compared with those seen in adults) do not lead to as rapid a decline in CD4+ T cell numbers [6].

Autopsy studies, SCID-hu chimeric mouse experiments, and indirect measures of thymic function all indicate that HIV may directly affect thymic output, thereby further limiting the replenishment of depleted CD4+ T cells [7–10]. In order to measure thymic function more directly in humans, we recently described an assay that quantifies the episomal DNA by-products of the T cell receptor (TCR) rearrangement process [11]. These TCR rearrangement excision circles (TRECs) contain the signal joint sequences from the TCRAD locus [12]. Here we confirm the effect of HIV upon the thymus. Indeed, this assay was recently used to show that HIV-infected children do have decreases in sjTRECs, which is suggestive of altered thymic function [12].

Patients and Methods

Patients. Children undergoing antiretrovirus combination therapy at the University of Massachusetts Pediatric HIV Program were chosen for study. All children had been infected with HIV from their mothers in utero or peripartum, but the timing of transmission could not be determined, as early postpartum blood
samples were unavailable. The antiretroviral regimens were chosen on the basis of prior treatment history and were altered, as necessary, in response to incomplete virus suppression. Age-matched control subjects were HIV-uninfected infants and children who were evaluated for possible HIV infection at the same institution. All children selected as controls were normal, healthy, and HIV unexposed, except for 1 child. This child was born to an HIV-infected mother but has never proved to be HIV positive, nor has this child manifested any abnormal immunologic traits.

**Virus and T cell measurements.** Plasma virus loads were determined by Roche Amplicor assay (Roche, Indianapolis), with a sensitivity of 400 RNA copies/mL. Percentages of CD4+ and CD8+ T cells were determined by flow cytometry according to the AIDS Clinical Trials Group (ACTG) consensus protocol. Peripheral blood mononuclear cells (PBMC) were also separated by density centrifugation and were cryopreserved in 10% dimethyl sulfoxide solution by use of a rate-controlled cell freezer; cells were then stored in liquid nitrogen according to the ACTG consensus protocol.

**Measurement of thymic function.** The frequency of sjTREC was quantified by quantitative-competitive polymerase chain reaction (QC-PCR) [11]. Cryopreserved PBMC were separated into CD4+ and CD8+ populations with MACS CD4 and CD8 magnetic microbeads and positive selection columns (Miltenyi Biotech, Auburn, CA). Cells were lysed in 100 µg/mL proteinase K (Boehringer Mannheim, Indianapolis) for 1 h at 56°C and then for 10 min at 95°C at a concentration of 10^6 cells/100 µL. Ten microliters of the cell lysate (equivalent to 100,000 cells) was used in each PCR reaction, to which was added 10^4, 10^3, or 10^2 molecules of internal standard, and QC-PCR was carried out as reported elsewhere [11]. Briefly, PCR products were separated on nondenaturing 6% polyacrylamide gels. Bands were imaged and analyzed with a Cyclone phosphorimager and Optiquant software (Packard Instruments, Meriden, CT). To quantify the number of TREC molecules in the reaction, the target TREC band intensity was divided by the corrected standard band intensity and multiplied by the number of standard molecules in the reaction.

**Statistical analysis.** TREC levels were compared between the HIV-infected and HIV-uninfected groups and between pre- and posttreatment time points by use of a 2-tailed equal variance t test performed with Microsoft Excel software.

**Results**

Nine HIV-infected children, ages 4 months–12 years, were studied. They were treated with combinations of 2 nucleoside reverse-transcriptase inhibitors (zidovudine, didanosine, stavudine, or epivir), with or without the addition of a nonnucleoside reverse-transcriptase inhibitor (nevirapine) and/or a protease inhibitor (nelfinavir or ritonavir). The frequency of sjTREC within the CD4+ T cells from these 9 patients was determined from PBMC samples obtained prior to the initiation of potent antiretroviral therapy, and this frequency was compared with that of 17 HIV-uninfected age-matched controls. As shown in figure 1, the 9 HIV-infected children had low frequencies of sjTREC within their CD4+ T cells when compared with HIV-uninfected controls (P < .001). This was true even in the 2 infants who were examined 3 and 4 months after birth, which indicates that the decrease in sjTREC can occur relatively early in the course of HIV infection in infants. A similar decrease in sjTREC was found in the CD8+ T cell subset (data not shown), and this decrease indicates a probable direct effect of HIV on thymic function rather than expansion or death of peripheral CD4+ T cells.

All 9 patients were treated with multidrug regimens of antiretroviral therapy. Of the 9 patients, 5 had suppression of viral replication to <400 copies/mL (and this value was lower in those who were tested with the ultrasensitive assay), whereas 4 had transient or incomplete suppression of viral replication (figure 2A). All children experienced substantial increases in total peripheral CD4+ T cell numbers, and there was no statistically significant difference in the rate of CD4+ T cell increase between the virologic responders (virus load suppressed to <400 copies/mL) and the nonresponders (virus loads remained ≥400 copies/mL; figure 2B). Therefore, there was no apparent benefit to total CD4+ T cell reconstitution as a result of potent virus suppression. However, there was a difference in the recovery of sjTREC frequency between virologic responders and nonresponders (figure 2C). Four of the 5 virologic responders had increases in sjTREC within their peripheral CD4+ T cell pool, whereas only 1 of the 4 virologic nonresponders had a sustained...
Figure 2. Plasma human immunodeficiency virus (HIV) levels, CD4\(^+\) T cell percentages, and T cell receptor rearrangement excision circle (TREC) levels after treatment with antiretroviral therapy. The 9 HIV-infected children were divided into virologic responders (left panels) or virologic nonresponders (right panels) on the basis of whether their plasma virus loads reached and were maintained at \(<400\) copies/mL (A). Each symbol represents an individual patient, with time point 0 being the day potent antiretroviral therapy was initiated. A, Age (months) of each subject at time point 0 and treatment initiated (a, zidovudine; b, didanosine; c, epivir; d, stavudine; e, nevirapine; f, nelfinavir; g, ritonavir). B, Increases over time in the percentages of CD4\(^+\) T cells in peripheral blood. C, Changes over time in sjTREC per 100,000 CD4\(^+\) T cells.

Discussion

The high activity of the thymus in children could represent both an advantage and a detriment to the HIV-infected child. An active thymus may offer more target T cells for HIV to infect (as opposed to the involuted thymus of an adult), thereby leading to more rapid and profound immunodeficiency. Alternatively, if the thymus is not directly affected by HIV, the greater output of naïve T cells from the thymus may be a factor that helps compensate for the CD4\(^+\) T cell loss from the very high virus loads that have been reported in HIV-infected infants [6].

There is mounting evidence that a defect in T cell production contributes to the inability of the immune system to compensate for the losses in CD4\(^+\) T cells as a result of HIV infection [1]. Autopsy studies and experiments that utilize fetal thymic tissue implanted into SCID mice confirm that HIV can infect human thymocytes and lead to their destruction [7–10]. Newer assays of thymic output that measure the frequency of sjTREC in PBMC also suggest that there is a decrease in T cell production from the thymus in HIV-infected infants and adults [11, 12]. The prior report in children had measured sjTREC frequencies in total PBMC [12]. Our data in 9 HIV-infected infants clearly demonstrate a decrease, in comparison with data associated with age-matched HIV-uninfected children, in sjTREC frequency in both the CD4\(^+\) and CD8\(^+\) T cell subsets. This decrease is apparent if sjTREC frequency is calculated on a per-T cell or a per-naïve T cell basis. Two conclusions can be drawn. First, the decrease in sjTREC previously reported in PBMC is not simply a reflection of a decrease in total T cell number or of a shift in naïve T cell frequency within the PBMC fraction, but rather it reflects a true decrease in sjTREC frequency within thymus-derived cells. Second, because at least 2 of the infants were diagnosed and tested within 3–4 months of birth, our data indicate that sjTREC can decline fairly quickly after the onset of HIV infection.

The decrease in sjTREC with HIV infection could represent a decrease in thymic output, an increase in turnover or death of sjTREC-containing T cells after they emigrate to the thymus, or both. Although our data cannot definitively determine which mechanism is operative, the fact that there is loss of sjTREC in both CD4\(^+\) and CD8\(^+\) T cells, whereas HIV infection should lead to increased death of only CD4\(^+\) peripheral T cells, suggests that a decrease in thymic output is the predominant mechanism.

Treatment of HIV infection with combinations of antiretroviral agents is known to lead to increases in CD4\(^+\) T cell numbers in infants and children [13]. After initiation of treatment, all 9 of our patients showed increases in CD4\(^+\) T cell numbers.
which were predominantly composed of memory CD4\(^+\) T cells (data not shown; figure 2B). However, not all of the children had sustained suppression of viral replication (figure 2A). Increases in sjTREC levels were observed in many of the patients but were most apparent in those patients who had suppression of viral replication to <400 copies/mL plasma (figure 2C). This indicates that greater suppression of viral replication may be required for recovery of thymic function than for return of CD4\(^+\) T cells through alternative mechanisms, such as proliferation of existing memory or naïve CD4\(^+\) T cells [2, 3]. Because production of new naïve T cells via the thymus will allow for replacement of clones of T cells lost to HIV infection (whereas peripheral expansion of existing cells will not), restoration of thymic function should be a goal of treatment of HIV infection. Our data indicate that this occurs when viral replication is profoundly suppressed and thus provide a further reason to recommend treatment regimens that lead to undetectable levels of plasma viremia, even in older children with severe CD4\(^+\) T cell depletion.

In conclusion, our data strongly indicate that the thymus of an infant or child is adversely affected by HIV but that with adequate suppression of viral replication, there is recovery of thymic function that should aid in the overall T cell reconstitution of the patient.

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References