T cell turnover and thymic function in HIV-1 infection
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T cell dynamics in HIV-1 infected children: a longitudinal study on TREC s, T cell numbers and peripheral T cell division before and during antiretroviral therapy

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Abstract

Interference of HIV-1 with thymic function may, if present, be more easily demonstrable in HIV-1 infected children, because thymic output is higher in infants as compared to adults. Measurement of T cell receptor excision circles (TRECs) has been thought to allow quantitative analysis of thymic function. Apart from thymic output, TREC dynamics are however also determined by other factors such as T cell division and the longevity of T cells. Because it is not known to what extent pediatric T cell division rates are disturbed by HIV-1 infection, we combined analyses of naive and memory T cell numbers and TREC dynamics with T cell division rates longitudinally in a group of HIV-1 infected children of different ages before and during antiretroviral therapy. In healthy and HIV-1 infected children, T cell division appeared to be highly dependent on age. Analyses of TRECs and T cell numbers suggested early interference of HIV-1 with the establishment of the T cell pool, but did not provide evidence for, nor excluded, ongoing thymic dysfunction. Antiretroviral therapy led to an improvement of all parameters, but in children older than 2 years, naive CD4$^+$ T cell numbers failed to normalize. This suggests that also the pediatric thymus is not capable to increase its output upon higher demands.
Introduction

It has been hypothesized that interference of HIV-1 with T cell renewal may contribute to the loss of CD4⁺ T cells that characterizes HIV-1 infection (1-5). As thymic output of naive T cells is highest in children, and infants have higher plasma HIV-1 RNA levels compared to adults (6), interference of HIV-1 with thymic function should be readily demonstrable in children. On the other hand, only one-fifth to one-fourth of perinatally HIV-1 infected patients experience early, severe immunodeficiency and rapid disease progression, whereas the majority of pediatric patients has survival times that do not differ from those observed in adults (7-9), despite higher viral loads (6). This could be related to better thymic function in children compared to adults.

Several groups investigated thymic function in HIV-1 infected infants, for example by magnetic resonance imaging (MRI) assessment of thymic volume. In a cross-sectional analysis of 31 vertically infected children, thymic volume correlated with the percentage and absolute number of naive T cells and with proliferative capacity to antigenic stimuli of PBMC: a negative correlation was found between thymic volume and plasma HIV-1 RNA (10). Highly active antiretroviral therapy (HAART) led to a rise in the proportion of naive T cells accompanied by an increase in thymic volume (11), however, the increase of naive T cell numbers and of thymic size correlated poorly.

In more recent studies thymic output in HIV-1 infected children was estimated with the detection of T cell receptor excision circles (TRECs) (12-16). TRECs are episomal products that are formed during T cell receptor (TCR) gene rearrangements that characterize intrathymic T cell development. Cross-sectional and longitudinal analyses of TRECs in perinatally infected children showed low TRECs content levels, suggesting decreased thymic function, that could be restored in infants who responded to antiretroviral therapy (12-14). In one study HAART did not induce significant changes of average peripheral blood mononuclear cell (PBMC) TREC content despite effective virus suppression and rising CD4⁺ T cell numbers (15).

In a previous study where we analyzed TREC dynamics in HIV-1 infected adults we found that naive CD4⁺ and CD8⁺ T cell TREC content is influenced by increased T cell proliferation levels characteristic of HIV-1 infection (17). Indeed, TRECs are episomal circles that are diluted upon cell division, which should thus be taken into consideration in the interpretation of TREC dynamics (12,18). It is however not known to what extent peripheral T cell division rates are increased in pediatric HIV-1 infection, and in addition, age may significantly influence T cell and TREC dynamics.

In the present study we analyzed changes in total, naive and memory CD4⁺ T cell numbers, TREC numbers, CD4⁺ T cell TREC content and peripheral total, naive and memory CD4⁺ T cell division rates in healthy children and in a group of HIV-1 infected children, before and during treatment with antiretroviral therapy. This resulted in several unexpected findings. In healthy children, peripheral CD4⁺ T cell
division rates correlated negatively with age, and pediatric HIV-1 infection was characterized by even higher T cell division rates, in addition to decreased TREC content and TREC numbers. Increased cell division and disturbed TREC dynamics were reversed by antiretroviral therapy. Nevertheless, in children older than two years naive CD4+ T cell loss failed to normalize suggesting that the thymus is not capable to homeostatically increase its output.

Methods

Subjects
Cryopreserved peripheral blood samples from 16 HIV-1 infected children and 29 healthy subjects of comparable ages (Table 1) were analysed. Cryopreservation was performed using a computerised freezing device that results in optimal quality of frozen cells for functional studies (19), and frozen blood samples were stored in liquid nitrogen. Samples were obtained before and at different intervals during treatment with triple therapy regimens that contained at least two reverse transcriptase inhibitors (nucleoside analogues) and one protease inhibitor. All except one of these children were vertically infected; this one child (M13388: 8.5 years of age in Figure 1) did not differ from the other children in T cell numbers, Ki67 expression or TREC dynamics.

Table 1. Baseline characteristics of untreated HIV-1 infected and healthy children

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 29)</th>
<th>HIV-1 infected (n = 16)</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.5 (0.3-16.0)</td>
<td>4.1 (0.3-12.9)</td>
<td>ns</td>
</tr>
<tr>
<td>CD4+ T cells (per μl)</td>
<td>1512 (400-3110)</td>
<td>7.1 (50-2650)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ki67+ CD4+ T cells (%)</td>
<td>2.6 (0.9-12.4)</td>
<td>10.2 (3.1-16.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TREC content (copies/CD4+ T cell)</td>
<td>0.095 (0.0002-0.458)</td>
<td>0.051 (0.013-0.187)</td>
<td>ns</td>
</tr>
<tr>
<td>Number of TRECs (per μl)</td>
<td>155.8 (14.7-1423.4)</td>
<td>43.5 (5.5-268.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (copies/ml)</td>
<td>na</td>
<td>2.4x10^3 (410-1.4x10^5)</td>
<td>na</td>
</tr>
</tbody>
</table>

Median values (and range) are depicted; p<0.05 was considered statistically significant (Mann-Whitney U Test); ns: not significant; na: not applicable.
Peripheral blood T cell subsets and T cell division

Peripheral blood T cell division was studied by flow cytometric measurements of Ki-67 nuclear antigen expression on naïve (CD27⁺/CD45RO⁻), CD27⁻ memory and CD27⁻ memory (CD27⁻ or CD27⁺CD45RO⁺) CD4⁺ T cells, as described previously (20-22). Peripheral blood mononuclear cells were thawed and incubated with CD4-PerCP monoclonal antibodies (mAb), CD45RO-PE (Becton Dickinson (BD), San Jose, California) and biotinylated CD27 mAb (CLB, Amsterdam, The Netherlands), washed, and incubated with Streptavidin-APC (BD). After fixation and Permeabilization with FACS Lysing Solution and FACS Permeabilization Buffer (BD), respectively, lymphocytes were stained intracellularly with Ki-67-FITC mAb (Immunotech, Marseille, France), fixed using Cellfix (BD) and analyzed on a FACSCalibur (BD) with Cellquest software.

Cell separation

CD4⁺ T cells were purified from thawed PBMC by magnetic separation over columns, using the MiniMACS multisort kit according to manufacturer’s instructions (Miltenyi Biotec Inc, Sunnyvale, California). Briefly, after 15 minutes incubation with 20 µl CD4 conjugated magnetic beads per 10⁶ cells, CD4⁺ T cells were isolated from PBMC by positive selection over MiniMACS separation columns. With this technique, at least 90% purity of CD4⁺ T cell fractions was achieved.

TREC analysis

DNA was purified from CD4⁺ T cells using the QIAamp Blood Kit according to manufacturer’s instructions (Qiagen, Hilden, Germany). Sj TREC content of these fractions was quantified by using a real-time PCR method (23) as described previously (17). To normalize for input DNA, the Ca constant region that remains present on TCR genes despite TCR rearrangement processes was amplified in every sample tested. The number of Sj copies present in a given cell population was calculated by including dilution series of a standard that was created by cloning the Sj fragment in the Hind III site of a plC-19 vector in each PCR experiment. By applying the Ct-value (the minimal number of cycles necessary to exceed threshold values) to the standardization curve the Sj TREC content could be calculated for each sample. TREC content was expressed as the number of TREC copies per CD4⁺ T cell, assuming that one µgram of DNA represents 150,000 T cells. The number of TRECs per µl blood was calculated by multiplying this TREC content with the number of peripheral blood CD4⁺ T cells (per µl).

Plasma HIV-1 RNA

Plasma HIV-1 RNA was measured using the Amplicor HIV-1 Monitor Test (Roche Diagnostic systems, Branchberg, New Jersey, USA), by the NASBA assay (Nuclisens HIV-1 RNA; Organon Teknika, Boxtel, The Netherlands) or by the Quantiplex bDNA test (Bayer, Mijdrecht, The Netherlands).
Chapter 9

Statistical analysis
Group characteristics of patients and healthy children were compared with the Mann-Whitney U test and correlations were calculated using Spearman's rank correlation coefficient (r).

Results

Baseline characteristics
Untreated pediatric HIV-1 infection was associated with significantly lower numbers of CD4+ T cells, due to a decline in naive and CD27+ memory but not CD27+ memory CD4+ T cells (Table 1 and data not shown; Figure 1). The proportion of Ki67+ CD4+ T cells was increased, which was related to a significant rise in the proportion of Ki67 naive, CD27+ and CD27+ memory CD4+ T cells (Table 1 and data not shown). Of note, the proportion of Ki67+ T cells in HIV-1 infected and in healthy children was much higher than observed previously in adults (median values ± standard deviation (SD)) for HIV+ adults 7.0 (± 4.7) % Ki67+ CD4+ T cells and for healthy adults 1.0 (± 0.6) % Ki67+ CD4+ T cells (20). In addition, the number of TREC was significantly lower in HIV-1 infected children but the CD4+ T cell TREC content (average number of TREC per CD4+ T cell) was not different from that of healthy age-matched controls (Table 1).

Effects of age on T cell numbers, T cell division rates, TREC numbers and TREC content
In healthy children, CD4+ T cell number, TREC number and CD4+ T cell TREC content correlated negatively with age (Figure 1 and Table 2). Interestingly, the proportion of Ki67+ CD4+ T cells also decreased with age (Figure 1 and Table 2). In HIV-1 infected children, similar associations with age were observed for CD4+ T cell numbers and for the proportion of Ki67+ CD4+ T cells, however, correlations between CD4+ T cell TREC content or the number of TREC and age were lost in HIV-1 infection (Figure 1 and Table 2). Interestingly, CD4+ T cell TREC content was significantly lower in HIV-1 infected children under 6 years but not in older patients compared with values obtained from age-matched healthy children (Table 3 and Figure 1c). The same was observed for the number of TREC (Table 3 and Figure 1d). Differences in CD4+ T cell numbers and the proportion of Ki67+ CD4+ T cells were also less pronounced between HIV+ and healthy children older than 6 years of age compared with differences observed between HIV+ and healthy children younger than 6 years, but they remained significant (Table 3 and Figure 1).

Plasma HIV-1 RNA was lower in children older than 6 years of age compared to younger HIV+ patients (medians values (±SD) 4.38 (±1.53) and 5.28 (±5.69) log copies/ml, p=0.08), but no significant association between HIV-1 plasma RNA and age was observed (Table 2). In addition, no correlations were found between HIV-
T cell dynamics in HIV-1 infected children

1 plasma RNA and the number of CD4⁺ T cells, the proportion of Ki67⁺ CD4⁺ T cells or CD4⁺ T cell TREC content (data not shown).

**Correlation between naive T cell numbers and TREC number or TREC content**

TREC content and TREC numbers are thought to allow direct assessment of thymic output. Indeed, in healthy children, the number of phenotypically naive CD4⁺ T cells correlated significantly with the number of TRECs and with the average CD4⁺ T cell TREC content (Table 4). However, in HIV-1 infected children the association between naive T cell number and CD4⁺ T cell TREC content was lost (Table 4), even in partial correlation analyses where we corrected for plasma HIV-RNA, the proportion of Ki67 expressing CD4⁺ T cells, or age (data not shown). The number of naive CD4⁺ T cells remained significantly associated with the number of TRECs (Table 4).

**T cell reconstitution during anti-retroviral therapy**

In all except one of the children under study, antiretroviral treatment led to an immediate decline in HIV-1 plasma RNA values, an improvement of CD4⁺ T cell numbers to normal ranges within 12 weeks and normalization of Ki67 expression on CD4⁺ T cells within 6 months (Figure 2). The median CD4⁺ T cell TREC content, that was not significantly lower at baseline than observed in healthy

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**Figure 1.** (a) T cell numbers, (b) Ki67 expression, (c) the average CD4⁺ T cell TREC content and (d) TREC numbers in HIV-1 infected and healthy children in relation to age. Black circles indicate healthy children (n=29), gray circles HIV-1 infected infants (n=16). For correlations between parameters and age, see Table 2.
Table 2. Correlations between age and T cell subset distributions, Ki67 expression, TREC number, CD4+ T cell TREC content and plasma HIV-1 RNA in healthy and untreated HIV-1 infected children

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>HIV-</th>
<th></th>
<th>HIV+</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r_s</td>
<td>p_s</td>
<td></td>
<td>r_s</td>
<td>p_s</td>
</tr>
<tr>
<td>CD4+ T cells (per µl)</td>
<td>-0.715</td>
<td>0.006</td>
<td>-0.719</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>- naive CD4+ T cells</td>
<td>-0.699</td>
<td>&lt;0.001</td>
<td>-0.763</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>- CD27+ memory CD4+ T cells</td>
<td>-0.706</td>
<td>0.015</td>
<td>-0.499</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>- CD27- memory CD4+ T cells</td>
<td>-0.592</td>
<td>ns</td>
<td>-0.592</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>Ki67+ CD4+ T cells (%)</td>
<td>-0.361</td>
<td>&lt;0.001</td>
<td>-0.667</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>- naive Ki67+ CD4+ T cells</td>
<td>-0.493</td>
<td>0.009</td>
<td>-0.979</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>- CD27+ memory Ki67+ CD4+ T cells</td>
<td>-0.686</td>
<td>0.041</td>
<td>-0.674</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>- CD27- memory Ki67+ CD4+ T cells</td>
<td>-0.393</td>
<td>ns</td>
<td>-0.537</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>TREC content (copies/CD4+ T cell)</td>
<td>-0.685</td>
<td>&lt;0.001</td>
<td>0.064</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Number of TREC s (per µl)</td>
<td>-0.794</td>
<td>&lt;0.001</td>
<td>-0.434</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>HIV plasma RNA (copies/ml)</td>
<td>na</td>
<td>na</td>
<td>-0.151</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

na: not applicable; ns: not significant (p < 0.05)

Figure 2. Rapid normalization of total CD4+ T cell numbers, Ki67 expression, the average CD4+ T cell TREC content and TREC numbers following introduction of HAART, in parallel with a reduction in plasma HIV-1 RNA.

Median values and standard deviations are shown for all HIV-1 infected (gray symbols) and healthy (black symbols) children. Asterix denotes value significantly different from control value (Mann-Whitney Test, p<0.05).

children, did not change in the course of treatment. The median number of TREC s that was however significantly lower at baseline, was restored within 3 months after initiation of HAART (Figure 2).
Table 3. Effect of age on TREC numbers, TREC content, cell division and CD4+ T cell numbers. Depicted are median values and ranges.

<table>
<thead>
<tr>
<th>Younger than 6 years</th>
<th>HIV-</th>
<th>HIV+</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREC content (copies/CD4+ T cell)</td>
<td>0.136 (0.07-0.46)</td>
<td>0.058 (0.01-0.18)</td>
<td>0.003</td>
</tr>
<tr>
<td>Number of TREC (per μl)</td>
<td>248 (96-1423)</td>
<td>49 (1-269)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD4+ T cells (per μl)</td>
<td>1753 (1000-3110)</td>
<td>850 (50-2650)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ki67+ CD4+ T cells (%)</td>
<td>3.0 (1.3-12.4)</td>
<td>11.0 (7.6-15.8)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Older than 6 years</th>
<th>HIV-</th>
<th>HIV+</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREC content (copies/CD4+ T cell)</td>
<td>0.040 (0-0.10)</td>
<td>0.044 (0.03-0.17)</td>
<td>0.254</td>
</tr>
<tr>
<td>Number of TREC (per μl)</td>
<td>40.1 (14.7-198.3)</td>
<td>13.5 (7.2-83.5)</td>
<td>0.200</td>
</tr>
<tr>
<td>CD4+ T cells (per μl)</td>
<td>965 (400-1892)</td>
<td>400 (300-630)</td>
<td>0.003</td>
</tr>
<tr>
<td>Ki67+ CD4+ T cells (%)</td>
<td>2.2 (0.9-10.5)</td>
<td>7.6 (3.1-16.7)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

As indicated above, T cell dynamics are highly dependent on age and in a previous study it was shown that only children of less than 2 years of age reached CD4+ T cell numbers that were considered normal for their age (25). When we excluded children younger than 2 years from our analyses, we indeed observed slower reconstitution dynamics, such that normal total CD4+ T cell numbers were only observed after 6 months of antiretroviral therapy (data not shown). Interestingly, complete naive CD4+ T cell reconstitution was never reached during the period under study. Even two years after initiation of HAART, HIV+ children, who were at least 2 years of age when antiretroviral therapy was started, had significantly lower naive CD4+ T cell numbers compared to age-matched healthy children (median values (± SD) 567 (± 233) and 1010 (± 539) naive CD4+ T cells/μl, p=0.034; Figure 3a). The one patient who virologically failed to HAART did nevertheless experience an increase in CD4+ T cell numbers. Indeed, when we excluded this patient from our analysis, naive CD4+ T cell numbers were still

Table 4. Correlation between naive CD4+ T cell number and TREC number or CD4+ T cell TREC content for healthy and HIV-1 infected children.

<table>
<thead>
<tr>
<th>Naive CD4+ T cell number (per μl)</th>
<th>HIV-</th>
<th>HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREC content (copies/CD4+ T cell)</td>
<td>r,</td>
<td>p,</td>
</tr>
<tr>
<td>Number of TREC (per μl)</td>
<td>0.564</td>
<td>0.006</td>
</tr>
<tr>
<td>r,</td>
<td>0.213</td>
<td>ns</td>
</tr>
<tr>
<td>p,</td>
<td>0.768</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ns: not significant (p &gt; 0.05).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3. Incomplete recovery of naive CD4+ T cell numbers in HIV-1 infected children older than 2 years on HAART (a). (b) In these children, the number of TREC recovered within 3 months and (c) the average CD4+ T cell TREC content did not change considerably during HAART. Black symbols indicate control values (n=19) and gray symbols HIV-1 infected patients (n=10) older than two years.

significantly lower 2 years after initiation of HAART compared with reference values (data not shown).

In children older than 2 years the number of TREC had recovered within 3 months (median values (± SD) for HIV+ and healthy children 65.0 (± 83.5) TREC/μl and 112.1 (± 124.9) TREC/μl, p>0.05, respectively: Figure 3b), in parallel with a decline in the proportion of Ki67+ CD4+ T cells (median values (± SD) for HIV+ and healthy children 4.9 (± 2.1) and 2.5 (± 2.3) % Ki67+ CD4+ T cells, p>0.05, respectively: data not shown). TREC content was not significantly lower at baseline and did not increase significantly during HAART (Figure 3c).

Discussion

In healthy children, the number of naive CD4+ T cells correlated significantly with the average CD4+ T cell TREC content and with the number of TREC, which suggests that these parameters all reflect thymic output (13-15,26). However, the average CD4+ T cell TREC content does not only depend on thymic production of TREC containing T cells, but also on peripheral T cell division, death and the longevity of naive T cells (17). In the present study we showed that in healthy children, peripheral CD4+ T cell division correlated inversely with age. HIV-1 infected children appeared to have even higher T cell division rates. The correlation between the number of naive CD4+ T cells and CD4+ T cell TREC content in these children was lost. These differences in correlations between healthy and HIV-1 infected children, and between children and adults, show that age is a very important parameter in the interpretation of pediatric T cell and TREC dynamics.
In our group of untreated HIV-1 infected children, the number of naive CD4⁺ T cells, the number of TRECcs and, in children younger than 6 years, the average CD4⁺ T cell TREC content were significantly lower compared to age-matched healthy individuals. As naive T cells are generally long-lived (in the order of years (27)), and TRECcs assumed to have a long half-life (24), complete abrogation of thymic function in adults is not expected to induce a rapid decline in naive T cell and TREC numbers (17,28). In children, however, who have higher thymic output, abrogation of thymic output has a dramatic impact when inflicted during active establishment of the T cell pool. This may be highly dependent on age, as involution of thymic tissue has been shown to be continuous, starting from birth (29). Indeed, early thymectomy, in the first year of life, led to significantly lower numbers of CD4⁺ T cells when measured 4–5 years later, whereas thymectomy in children older than one was not associated with a reduction of CD4⁺ T cell numbers (30). Due to the longevity of naive T cells low numbers of naive CD4⁺ T cells and TRECcs in our group of HIV-1 infected children does not simply reflect thymic dysfunction at the moment of analysis, but may be related to abrogation of thymic function in the first year of life, thereby interfering with establishment of the naive T cell pool. In two separate studies, HIV-negative children born from HIV-1 infected mothers were shown to have reduced numbers of naive CD4⁺ T cells, lower numbers of red blood cells and suppressed T cell progenitor function as measured in fetal thymic organ culture assays (26,31).

In our group of untreated HIV-1 infected children less than 6 years of age TREC content was significantly reduced, which can be attributed to the highly increased peripheral T cell division rates that we observed. Children older than 6 showed normal TREC content values. In adults, this is associated with slower disease progression (32). These children could therefore represent long-term non progressors, however, CD4⁺ T cell numbers were significantly lower, indicating that also in these children T cell dynamics were disturbed by HIV-1 infection. The proportion of Ki67⁺ CD4⁺ T cells in this subset of HIV-1 infected children was only slightly increased. Persistently increased proliferation of CD4⁺ T cells is expected to lower TREC content, but this dilution can be counteracted by increased T cell death rates and/or continuous thymic output of TREC containing T cells. Thus, those HIV-1 infected children older than 6 years may have preserved thymic function, keeping TREC levels up, although not enough to completely compensate for the increased loss of naive T cells.

Antiretroviral therapy led to a rapid improvement of TREC content and TREC numbers, although in children older than 2 years, the number of phenotypically naive T cells never reached normal values during the period under study (maximal follow up 2 years). Of note, normalization of TREC content and TREC numbers may involve redistribution of TREC containing naive and memory T cells from lymphoid tissues, reduction of T cell death and division rates and continuous or improving thymic output. Interestingly, in children older than 2 years of age, the number of naive CD4⁺ T cells increased but did not normalize, even after 2 years of effective HAART. In fact, it is not known whether normal naive T cell numbers
will ever be reached. In adult HIV-1 infected patients, naive CD4\(^+\) T cell numbers remained below control values after two years of HAART (33,34). In another study it was shown that the relatively few patients who survived 20 to 30 years after bone marrow transplantation had significantly lower numbers of naive CD4\(^+\) T cells, despite normalization of total CD4\(^+\) T cell counts (35). This suggests that thymic output in adults is low and is not significantly increased to compensate for low naive T cell numbers. Children are generally considered to reconstitute normal T cell numbers faster because of higher daily thymic output (36). However, when strictly related to age-matched reference values, reconstitution of naive T cell numbers in children following bone marrow transplantation or during HAART was as slow as observed in adults (25,37). It seems therefore that even in children recuperating from T cell depletion, thymic output remains rather constant, albeit at higher levels compared to adults.

Finally, whereas the number of naive CD4\(^+\) T cells remained low, the proportion of dividing naive CD4\(^+\) T cells that was significantly increased during untreated HIV-1 infection rapidly declined during HAART. This is in agreement with previous observations in HIV-1 infected adults receiving antiretroviral therapy and in oncology patients who received stem cell transplantation (20,38-40), and shows that also in HIV-1 infected children increased T cell division during T cell depletion is not related to T cell recovery.

Taken together, our data show that pediatric HIV-1 infection is characterized by increased peripheral CD4\(^+\) T cell division rates that were highly dependent on age and normalized rapidly during HAART. Because of the longevity of naive T cells and the complex interactions between age, cell division rates and TREC dynamics, it was not possible to demonstrate (or exclude) HIV-1 induced impairment of thymic output. Our data do however suggest early interference of HIV-1 with the establishment of the naive T cell pool. Although naive CD4\(^+\) T cell numbers increased with antiretroviral therapy in children older than 2 years of age, they did not normalize. This suggests that the thymus may not be capable to significantly increase its output to compensate for low naive T cell numbers. It therefore remains to be determined whether with time naive T cell loss can be truly overcome.

References

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