Long-Term Decay of the HIV-1 Reservoir in HIV-1–Infected Children Treated with Highly Active Antiretroviral Therapy

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To investigate the decay of the human immunodeficiency virus type 1 (HIV-1) reservoir in children receiving highly active antiretroviral therapy (HAART), we measured HIV-1 DNA in peripheral blood mononuclear cells from 14 children who achieved and maintained suppression of plasma viremia up to 48 months after the initiation of HAART. Levels of intracellular unspliced and multiply spliced HIV-1 RNA were used as markers of residual viral replication. During the first month of HAART, there were significant decays in levels of both plasma HIV-1 RNA and multiply spliced HIV-1 RNA, yet unspliced HIV-1 RNA persisted in most of the children. Greater HIV-1 DNA decay during the first month of HAART correlated with a higher concomitant increase in CD4+ cell counts (P = .028) and a smaller subsequent HIV-1 DNA decay (P = .0012). Furthermore, HIV-1 DNA decayed faster from 1 to 9 months of HAART (median half-life, 5 months) than during the subsequent follow-up period (median half-life, 30 months). Moreover, after 9 months of HAART, HIV-1 DNA tended to decay more slowly in children with detectable levels of unspliced HIV-1 RNA. These findings suggest that clearance of the viral reservoir in HAART-treated children may be influenced by immune repopulation and residual viral replication and may help in refining long-term treatment strategies.

Highly active antiretroviral therapy (HAART) has reduced HIV-1 disease progression and mortality in children and adults [1, 2], but it cannot eradicate the virus. Sensitive assays have demonstrated that resting memory CD4+ cells retain replication-competent viral DNA that may be reactivated to produce virus even in patients with prolonged suppression of plasma viremia [3]. In addition, ongoing low levels of viral transcription and replication may occur in a fraction of patients, despite suppression of plasma viremia, as has been demonstrated by the detection of cell-associated HIV-1 RNA [4, 5] and the evolution of viral sequences [6, 7].

Because an understanding of the clearance of viral reservoirs in HIV-1–infected individuals may help in refining long-term treatment strategies, clearance kinetics of virus populations have been extensively studied in adults who have achieved and maintained plasma virus suppression with HAART. By using limiting dilution cocultures to quantify cellular reservoirs of potentially infectious HIV-1, the half-life of the CD4+ cell latent reservoir was estimated to be 6 months in a cohort that included both chronically and acutely infected patients [8], but longer half-lives have been reported in adults who initiated HAART during chronic infection [9]. Ongoing residual replication of HIV-1 may refill the latent reservoir and affect clearance kinetics [8]. The quantification of HIV-1 DNA levels may provide a more sensitive measurement of the size of the viral reservoir. Using this approach, researchers studying HIV-1–infected adults have estimated that, after the initial clearance of productively infected cells, HIV-1
HAART have a greater capacity for CD4+ cell recovery, likely differences between adults and children in both their virological populations can be problematic—several studies have outlined [15, 16]. The extrapolation of results from adult to pediatric patients with a short period of undetectable plasma viremia HAART are scarce, and, thus far, they have been limited to half-life of 104 weeks thereafter [14].

Studies of viral decay in HIV−infected children receiving HAART are scarce, and, thus far, they have been limited to patients with a short period of undetectable plasma viremia [15, 16]. The extrapolation of results from adult to pediatric populations can be problematic—several studies have outlined differences between adults and children in both their virological and immunological response to therapy. Children receiving HAART have a greater capacity for CD4+ cell recovery, likely because of more-active thymopoiesis [15, 17−19], but they are also known to have a higher rate of incomplete suppression and/or rebound of HIV−1 RNA in plasma [20, 21]. This higher rate of virologic failure in children makes it difficult to study the long-term decay of viral reservoirs.

In the present study, we estimated clearance of the viral reservoir by measuring HIV−1 DNA in 14 chronically HIV−1−infected children who showed long-term control (up to 4 years) of HIV−1 replication while they were receiving HAART. Rates of clearance of HIV−1 DNA were compared with the dynamics of CD4+ cell recovery and residual viral replication as evaluated by the quantitative detection of intracellular HIV−1 RNA.

PATIENTS, MATERIALS, AND METHODS

Patient population. The study included 14 HIV−1−infected children born to HIV−1−seropositive Italian mothers and monitored prospectively by the Pediatric Department of Padova University since birth. All children were infected with clade B virus, as determined by sequences in the pol gene. The inclusion criterion was a prolonged virological response to protease inhibitor (PI)−based HAART, defined as suppression of plasma HIV−1 RNA to undetectable levels (<50 copies/mL of plasma) within 6 months of the initiation of HAART and persistent levels of <50 copies/mL of plasma thereafter, for at least 24 and up to 48 months. All children were PI naive at the initiation of HAART. Levels of proviral HIV−1 DNA and cell−associated HIV−1 RNA were evaluated in cryopreserved samples collected at the initiation of HAART and at fixed time points during follow−up.

Quantification of HIV−1 RNA in plasma. Plasma HIV−1 RNA levels were determined using the Roche Amplicor Monitor System (Roche). The lower limit of detection was 50 HIV−1 RNA copies/mL when the ultrasensitive protocol was used.

Quantification of HIV mRNA in cells. Total cellular RNA was extracted from peripheral blood mononuclear cells (PBMCs; 1 × 10^6−5 × 10^6), using the Trizol RNA extraction kit (Invitrogen), and resuspended in 30 μL of water that contained 0.01% diethylpyrocarbonate. Residual contaminating DNA was eliminated using the Deoxyribonuclease 1 kit (Invitrogen). Then, 24 μL of RNA was reverse transcribed into cDNA, using the Taqman Reverse Transcription assay (PE Applied Biosystems) in a final volume of 80 μL, in accordance with the manufacturer’s instructions. HIV−1 mRNA−unspliced and multiply spliced encoding tat/rev—was quantified by real−time polymerase chain reaction (PCR) using the following primers: US1 forward (5′−TTAGGTGTCTTCAATTGTGGGAAAAGA−3′, nt 1956−1981 in the HIVp22 genome), US2 reverse (5′−AAAGAGACAGGAGCTCTC−3′; nt 2059−2122), MS1 forward (5′−AAAGGAAAACACAGAGCTCT−3′; nt 672−693), and MS2 reverse (5′−GCCGCTGGGCTCCCTC−3′; nt 8438−8454) and the following probes: USP (FAM−5′−CCCGAGGGTGTGGAAATG−3′−TAMRA; nt 2007−2035) and MSP (FAM−5′−TCGACGGAGGACTCGGCCCTG−3′−TAMRA; nt 695−715). For the detection of unspliced RNA, the PCR assay was performed in a 50−μL final volume that contained 10 μL of cDNA, 25 μL of 2× Taqman Universal PCR Master Mix (PE Applied Biosystems), 300 nmol/L primer US1, 900 nmol/L primer US2, and 100 nmol/L probe USP. Amplifications were performed on a 96−well reaction plate (PE Applied Biosystems) in a thermal cycler (ABI Prism 7700; PE Applied Biosystems), using the following conditions: 2 min at 50°C, 10 min at 95°C, and 45 cycles each of 15 s at 95°C and 1 min at 60°C. For each run, a standard curve was generated from duplicate samples of 5−fold serially diluted known copies of the plasmid pBH10 [22]. Each sample was run in duplicate, and the mean threshold cycle (Ct) value was plotted against the standard curve to obtain the number of unspliced RNA copies in the sample. For the detection of multiply spliced RNA, the PCR assay was performed as described above, with the exception of the primer concentrations, which were 400 nmol/L for primer MS1 and 800 nmol/L for primer MS2. Samples were run in duplicate, and mean Ct values were plotted against the standard curve generated from a 5−fold dilution of an ampiclon. This ampiclon was obtained by amplifying cDNA obtained from 8E51 cells with the primers MS1 and MS2. Then, 12 μL of cDNA was amplified in a 50−μL final volume that contained 1× PCR Gold Buffer, 200 nmol/L each primer, 200 nmol/L dNTPs, 1.5 mmol/L MgCl2, and 2.5 U of Taq Gold (PE Applied Biosystems). Amplification was performed in a thermal cycler (9600 Perkin Elmer) for 35 cycles, each of which consisted of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C. The 242−bp ampiclon was run on 2% agarose gel, purified using...
Table 1. Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>CD4+ cell count (%), cells/µL</th>
<th>HIV-1 level</th>
<th>HIV-1 RNA level, log_{10} copies/1 × 10^7 copies of GAPDH</th>
<th>Treatment before HAART</th>
<th>HAART regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>220 (19)</td>
<td>4.13 3.31</td>
<td>3.47 2.49</td>
<td>None</td>
<td>3TC, D4T, RTV</td>
</tr>
<tr>
<td>A2</td>
<td>5</td>
<td>218 (11)</td>
<td>5.35 4.30</td>
<td>4.10 2.61</td>
<td>AZT, DDI</td>
<td>AZT, DDI, IDV</td>
</tr>
<tr>
<td>A3</td>
<td>11</td>
<td>70 (4)</td>
<td>4.35 2.08</td>
<td>3.04 2.15</td>
<td>AZT, 3TC</td>
<td>AZT, 3TC, RTV</td>
</tr>
<tr>
<td>A4</td>
<td>6</td>
<td>482 (19)</td>
<td>4.93 3.16</td>
<td>4.06 2.86</td>
<td>None</td>
<td>AZT, 3TC, NFV</td>
</tr>
<tr>
<td>A5</td>
<td>8</td>
<td>98 (2)</td>
<td>5.69 3.19</td>
<td>2.60 1.48</td>
<td>DDI, 3TC</td>
<td>D4T, 3TC, IDV</td>
</tr>
<tr>
<td>A6</td>
<td>8</td>
<td>530 (33)</td>
<td>5.42 3.48</td>
<td>3.09 2.40</td>
<td>AZT, DDI</td>
<td>AZT, 3TC, RTV</td>
</tr>
<tr>
<td>A7</td>
<td>10</td>
<td>342 (24)</td>
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<td>3.42 2.52</td>
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<td>AZT, 3TC, NFV</td>
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<td>60 (6)</td>
<td>3.97 1.90</td>
<td>&lt;1.40^a &lt;1.40^a</td>
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<td>AZT, 3TC, IDV</td>
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<td>2.00 1.40</td>
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<tr>
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<td>380 (9)</td>
<td>5.17 2.00</td>
<td>2.59 &lt;1.40^a</td>
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<td>5.02 2.85</td>
<td>2.30 2.11</td>
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<td>DDI, 3TC, NFV</td>
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</tbody>
</table>

NOTE. 3TC, lamivudine; AZT, zidovudine; D4T, stavudine; DDI, didanosine; HAART, highly active antiretroviral therapy; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir.

^a Below the limit of detection.

the NucleoSpin Extract kit (M-Medical), and quantified using spectrophotometry. Under these conditions, both assays were sensitive enough to detect 2.5 copies of HIV-1 RNA and showed a dynamic range of at least 5 log_{10}. To normalize HIV-1 RNA, 10 μL of cDNA of each sample was amplified by real-time PCR, under the conditions described above, for the housekeeping gene GAPDH, using the GAPDH assay (PE Applied Biosystems). The HIV-1 RNA copies were then expressed relative to copies of GAPDH.

HIV-1 DNA quantification. Quantitative determination of HIV-1 DNA was performed on lysed PBMCs [23] by real-time PCR, exactly as described elsewhere [15].

Immunophenotyping. CD4+, naive CD4+CD45+RA, and memory CD4+CD45+RO cell counts were evaluated by cytometry, as reported elsewhere [15]. Naive and memory cell counts were assessed only in a subset of children.

Statistical methods. Changes in levels of HIV-1 DNA, unspliced HIV-1 RNA, HIV-1 RNA in plasma, and CD4+ cells were estimated using standard normal linear regression, log_{10} transformed where appropriate, and adjusted for multiple measurements in individual children by use of robust regression [24]. Values below the limit of detection were analyzed using normal interval regression in which the undetectable value was replaced by the interval in which the true value could lie (i.e., the interval up to the cutoff) [25]. In view of significant differences in HIV-1 DNA decay rates during different periods after the initiation of HAART, we used piecewise linear models to describe these variations, with slope change points chosen after a visual inspection of mean values (see Results). The half-life of HIV-1 DNA was calculated using the formula \( t_{1/2} = -\log_{10} 2 / \text{slope} \). Spearman rank correlations were used to estimate pairwise associations.

RESULTS

Baseline characteristics of the study population. At baseline, the median age of the study population was 9 years (range, 3–14 years), the median CD4+ cell count was 219 cells/µL (range, 9–611 cells/µL), the mean ± SD plasma HIV-1 RNA level was 4.75 ± 0.59 log_{10} copies/mL, and the mean ± SD HIV-1 DNA level was 2.85 ± 0.66 log_{10} copies/1 × 10^7 cells (table 1). Intra-cellular unspliced and multiply spliced HIV-1 RNA was detectable in 13 and 12 children, respectively, at means ± SDs of 2.85 ± 0.76 and 2.00 ± 0.55 log_{10} copies/1 × 10^7 copies of GAPDH.

Virological changes after the initiation of HAART. HIV-1 RNA levels in plasma decayed by 1.73 log_{10} copies/mL (95% confidence interval [CI], 1.25–2.22 log_{10} copies/mL) and 2.83 log_{10} copies/mL (95% CI, 2.53–3.14 log_{10} copies/mL) at 1 and 3 months, respectively, after the initiation of HAART. Thereafter, HIV-1 remained undetectable in plasma (<50 copies/mL) throughout the follow-up period (figure 1A). HIV-1 DNA levels also decayed substantially by 0.17 log_{10} copies/1 × 10^7 cells (95% CI, 0.02–0.32 log_{10} copies/1 × 10^7 cells), 0.34 log_{10} copies/1 × 10^7 cells (95% CI, 0.16–0.52 log_{10} copies/1 × 10^7 cells), and 1.10 log_{10} copies/1 × 10^6 cells (95% CI, 0.78–1.43 log_{10} copies/1 × 10^6 cells).
Figure 1. Changes in HIV-1 and CD4+ cells after the initiation of highly active antiretroviral therapy (HAART). A, Change in no. of log_{10} HIV-1 RNA copies per milliliter of plasma (white squares) and log_{10} HIV-1 DNA copies per 1 × 10^6 cells (white circles). B, Changes in nos. of CD4+, naive CD4+CD45+RA, and memory CD4+CD45+RO cells per microliter. Mean values and 95% confidence intervals (CIs; vertical bars) are shown. Naive and memory cells were evaluated only in a subset of children; mean changes in CD4+ cell count in all children and in the subsets of children with and without naive/memory immunophenotyping were quite similar.

1 × 10^6 cells) after 1, 3, and 48 months of HAART, respectively (figure 1A). At the last follow-up, HIV-1 DNA was still detectable in all but 2 children (A3 and A13) (figure 2). Although no blips of plasma viremia were detected, a low level of residual viral replication cannot be excluded. We measured levels of intracellular HIV-1 RNA as markers of ongoing viral replication. In addition to unspliced HIV-1 RNA, the multiply spliced HIV-1 RNA encoding tat/rev plays a central role in the retroviral life cycle [26, 27], and its detection has been associated with newly infected cells [28–30]. We found that the decay of mul-
Figure 2. Kinetics of HIV-1 DNA, unspliced HIV-1 RNA (RNAus), and multiply spliced HIV-1 RNA (RNAms) in children. Viral parameters were estimated by real-time polymerase chain reaction at baseline and subsequently during highly active antiretroviral therapy. The identification codes of patients are reported within each panel.
The rate of HIV-1 DNA decay during HAART was not simply exponential—it was faster immediately after the initiation of therapy, which is in agreement with previous observations [11, 31]. The mean changes in HIV-1 DNA levels (figure 1A) suggested that the overall change from baseline could be approximated by a piecewise linear model that included a slope to 1 month (to allow for immediate redistribution effects), from 1 to 9 months, and from 9 months onward. When this model was used, the estimated overall rate of HIV-1 DNA decay per month was 0.258 log_{10} copies/10^6 cells (95% CI, 0.030–0.486 log_{10} copies/10^6 cells) in the first month of HAART, 0.054 log_{10} copies/10^6 cells (95% CI, 0.026–0.082 log_{10} copies/10^6 cells) from 1 to 9 months of HAART, and 0.008 log_{10} copies/10^6 cells (95% CI, 0.005–0.022) after 9 months of HAART, with decays in HIV-1 DNA level from 1 to 9 months and after 9 months differing significantly ($P = .005$). Although fitting this model to the data from each child separately demonstrated that the kinetics of HIV-1 DNA decay were quite heterogeneous, the means of individual child-specific slope were very similar to those obtained from the model, as expected (table 2). From 1 to 9 months of therapy, individual slopes of HIV-1 DNA decay ranged from 0.155 to 0.111 log_{10} copies/10^6 cells/month, with a mean value of 0.046 log_{10} copies/10^6 cells and a median value of 0.065 log_{10} copies/10^6 cells, corresponding to a mean (median) half-life of HIV-1 DNA decay of 7 (5) months. After 9 months of therapy, individual slope values ranged from 0.087 to 0.038 log_{10} copies/10^6 cells/month, with a mean value of 0.016 log_{10} copies/10^6 cells and a median value of 0.010 log_{10} copies/10^6 cells, corresponding to a mean (median) half-life of HIV-1 DNA decay of 19 (30) months. Furthermore, greater decays in HIV-1 DNA levels during the first month of HAART were followed by smaller decays during the subsequent period ($r = -0.771; P = .001$) (figure 3).

**CD4⁺ cell immune repopulation influence on short-term HIV-1 DNA decay.** CD4⁺ cell counts had increased by 518 cells/μL (95% CI, 366–670 cells/μL) at 24 months after the initiation of therapy, which is in agreement with previous observations [11, 31]. The mean changes in CD4⁺ cell counts (figure 2) were followed by smaller decays during the subsequent period ($r = -0.771; P = .001$) (figure 3).
Thereafter, the mean slope of the increase in CD4+ cell count was 0.673 log10 copies/cell/month during the first month of HAART (figure 4A and 4B). Because there is a natural but substantial decline in absolute CD4+ cell count in uninfected children during the first years of life, results were confirmed using age-adjusted CD4+ cell z scores [33] (figure 4A and 4B). Thereafter, no significant relationship was found between changes in CD4+ cell count and HIV-1 DNA decay (1–9 months, \( r = -0.279 \) and \( P = .338 \); >9 months, \( r = 0.134 \) and \( P = .648 \)).

**Influence of unspliced HIV-1 RNA decay on long-term HIV-1 DNA decay.** Unspliced HIV-1 RNA was either persistently or seldom detected during the first 9 months of HAART in all but 1 child, who tested negative at baseline. The mean slope of unspliced HIV-1 RNA decay per month was 0.673 log10 copies/1 \times 10^6 \) cells/month of therapy and 0.103 log10 copies/1 \times 10^6 \) copies of GAPDH (95% CI, 0.029–0.176 log10 copies/1 \times 10^7 \) copies of GAPDH) from 1 to 9 months of HAART. No significant relationship was observed between the decay of HIV-1 DNA and that of unspliced HIV-1 RNA during the first month or from 1 to 9 months of therapy in individual children. Three children (A2, A9, and A13) (figure 2) were persistently negative for unspliced HIV-1 RNA after 9 months of HAART. Although numbers were small, the rate of HIV-1 DNA decay after 9 months tended to be higher in these 3 children than in 11 children with detectable unspliced HIV-1 RNA after 9 months of HAART (mean decay, 0.048 [range, 0.087–0.006] vs. 0.007 [range, 0.032–0.037] log10 copies/1 \times 10^6 \) cells/month; \( P = .029 \), Student’s t test; \( P = .31 \), rank sum test).

**DISCUSSION**

The present article describes the decay of the HIV-1 reservoir in children with long-term sustained suppression of plasma viremia. We evaluated the HIV-1 reservoir by quantifying total HIV-1 DNA in PBMCs. Given the presence of defective genomes and linear and circular forms of unintegrated viral DNA [34, 35], these HIV-1 DNA levels may provide an upper limit for the number of infected cells that exceeds the number of replication-competent provirus genomes. Although the rate of HIV-1 DNA decay after the initiation of HAART varied widely from child to child, we found that the first phase of the to decay in HIV-1 DNA levels, during the first month, was more rapid than the subsequent rate of decay. This is in agreement with our previous observation [31] and with the notion that the clearance of most productive infected cells and labile linear preintegration forms of viral DNA occurs soon after the initiation of HAART [11, 36]. The observed rapid clearance of multiply spliced HIV-1 RNA, a surrogate marker of productive infected cells [37], supports this concept. This initial phase of HIV-1 DNA decay was influenced by the dynamics of increases in CD4+ cell counts. In particular, a large increase in CD4+ cell counts during the first month was associated with a concurrent large amount of HIV-1 DNA decay but with a less steep decay in HIV-1 DNA levels during the immediate follow-up period, from 1 to 9 months of HAART. During HAART, children have a greater and faster capacity for CD4+ cell recovery than adults,
mainly because of more active thymopoiesis, and they reconstitute CD4+ cells mainly with naive thymically derived cells [15, 18, 32], which HIV-1 infects poorly [38]. This rapid repopulation by newly uninfected cells is likely to initially dilute the number of HIV-1 DNA–positive cells. Nevertheless, during the first months of HAART, most children still had detectable, albeit decreasing, circulating infectious virus in plasma. In this milieu, circulating antigens may activate naive CD4+ cells to differentiate into memory cells, which are more susceptible to HIV-1 infection [39]. The increase in numbers of CD4+ cells suitable for new cycles of viral infection might negatively affect the subsequent HIV-1 DNA decay. This is in agreement with our previous observation that the decline in HIV-1 DNA levels was influenced negatively by the increase in thymically derived cells in children who did not achieve a sustained suppression of HIV-1 RNA in plasma during antiretroviral treatment [21].

After the initial rapid clearance of productive infected cells, the HIV-1 reservoir in patients with sustained suppression of plasma viremia is mainly composed of latently infected memory T cells [3, 9]. In adults, it has been estimated that this reservoir decayed with a median half-life of 4–6 months [8, 11, 12], which is close to the estimated rate of decay of memory T lymphocytes [10]; however, slower rates of decay have been reported in other studies [9]. All of these studies were performed under the assumption that clearance of the latent HIV-1 reservoir was constant over time. Recently, studies in adults treated with HAART for 3–4 years have indicated that HIV-1 DNA decays more slowly after 1 year (median half-life, 70 weeks) than during

Figure 4. A, Relationship between slopes of HIV-1 DNA decay and increases in CD4+ cell count during the first month of highly active antiretroviral therapy (HAART). B, Relationship between slopes of HIV-1 DNA decay from 1 to 9 months of HAART and increase in CD4+ cell count during the first month of HAART. Because there is a natural but substantial decline in absolute CD4+ cell counts in uninfected children during the first years of life, the relationship between HIV-1 DNA decay and increases in CD4+ cell count was determined using both the absolute CD4+ cell count and the age-adjusted CD4+ cell z score.
the first 1–12 months of therapy (median half-life, 20 weeks) [13]. In agreement with this result, we also observed that decay of the HIV-1 reservoir after the first month is not simply exponential—it is more rapid during the first 1–9 months of HAART (median half-life, 5 months) than after 9 months (median half-life, 30 months). This is consistent with the finding that cellular reservoirs contain cells that clear at different rates and with a progressive increase over time of more-stable, long-lived, latently infected cells to these reservoirs [40].

The rates of clearance of HIV-1 DNA that we observed in children were slower than those estimated in adults [8, 10–14]. Notably, an overall decay of HIV-1 DNA with a median half-life of 14 months was also reported in children with HIV-1 RNA suppression in plasma [15]. Several factors may influence the rates of clearance of the latent HIV-1 reservoir, including residual viral replication. Although HIV-1 RNA was undetectable in plasma in all measurements after its initial decline and multiply spliced HIV-1 RNA was rarely found during the first 9 months, and blips in levels of unspliced RNA were detected in 11 and 8 children after 9 and 12 months of therapy, respectively. Similar findings of residual unspliced HIV-1 RNA have been reported in both PBMCs [4, 5, 41] and lymphoid tissue [37] in patients receiving effective antiretroviral therapy, whereas RNA species encoding tat/rev were undetected. It cannot be excluded that low levels of multiply spliced HIV-1 RNA, below the detection limit, were present in samples, and the persistence of unspliced HIV-1 RNA might be viewed as a sign of an ongoing productive infection. The detection of unspliced HIV-1 RNA may also reflect ongoing cellular activation with an incomplete pattern of viral transcription and a lack of virion production. Stimulation by antigens may provide an opportunity for latently infected cells to become activated and to initiate the program of viral genome transcription. HIV-1–infected memory CD4+ cells that recognize common antigens may be preferentially activated and cleared during the first phase of latent HIV-1 reservoir decay, whereas other cells, including those that recognize uncommon antigens and other cell types [42–44], may be activated slowly and eventually eliminated at a slower clearance rate. Although further studies on separate cell subsets are needed to clarify the varying contributions of different cell types to the residual viral reservoir, we estimated that this residual reservoir was cleared with a half-life of 6–43 months. A slower rate of decay may be expected when unspliced HIV-1 RNA is present, which suggests that residual viral transcription and replication nearly balance viral clearance. These findings indicate that the eradication of HIV-1 infection is not feasible in chronically infected children. The results of our study should be useful in refining long-term therapeutic strategies.

Acknowledgments

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References