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Association of CD4⁺ T-lymphocyte counts and new thymic emigrants in HIV-infected children during successful highly active antiretroviral therapy

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Abstract

Background—In a cohort of children receiving highly active antiretroviral therapy (HAART) with sustained plasma HIV-1 RNA < 50 copies/mL, children who reached undetectable RNA after week 8 (slow responders, median: week 20) had higher HIV-1 intracellular DNA (HIV-1 DNA) and equal or greater CD4⁺ T-lymphocyte counts compared with children who reached undetectable plasma HIV-1 RNA by week 8 (rapid responders) throughout HAART.

Objective—To determine whether levels of T-cell receptor excision circles (TRECs) could explain the apparent inconsistency between the quantity of HIV-1 DNA and CD4⁺ T-lymphocyte counts in HIV-1–infected children receiving HAART with sustained virologic suppression.

Methods—T-cell receptor excision circles and HIV-1 DNA and plasma HIV-1 RNA were quantified longitudinally by PCR in 31 children (median age, 5.6 years) with sustained undetectable plasma HIV-1 RNA for >104 weeks of HAART.

Results—There was a positive correlation between TREC and HIV-1 DNA during HAART, notably at weeks 48 and 80 ($P < .004$). During the early stage of HAART, TREC levels positively correlated with CD4⁺ T-lymphocyte percentages ($P < .02$) and naive CD4⁺ T-lymphocyte counts ($P < .001$) and percentages ($P = .05$). Median TREC levels were consistently equal or higher in slow responders compared with rapid responders ($P < .001$) despite slow responders having consistently greater quantities of HIV-1 DNA.

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

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Conclusion—To maintain adequate levels of CD4⁺ T-lymphocytes, children with high HIV-1 DNA maintain high levels of TREC while receiving HAART. Thus, a thymic control mechanism is required to maintain new CD4⁺ T lymphocytes in the presence of persistent virus.

Clinical implications—The TREC level is a useful marker of thymic function in HIV-infected children.

Keywords

T-cell receptor excision circles; immune reconstitution; HIV-1 intracellular DNA; CD4⁺ T lymphocytes; HAART; children

HIV-1 infection is characterized by gradual depletion of circulating CD4⁺ T lymphocytes, resulting in progressive immunodeficiency that leads to opportunistic infections and death. The availability of highly active antiretroviral therapy (HAART) has changed the clinical course of HIV-1–infected children and adults dramatically with rapid declines in plasma HIV-1 RNA and an increase in CD4⁺ T-lymphocyte counts.^{1,2}

The thymus plays an important role in immune homeostasis and immune reconstitution.³ In HIV-1–infected children, the increase in CD4⁺ T lymphocytes associated with HAART is dominated by the replenishment of naive cells believed to result from the continued presence of a functional thymus.^{4–6} In adults, a biphasic recovery of CD4⁺ T lymphocytes has been described associated with a rapid increase of memory T lymphocytes, followed by a slow increase in naive T lymphocytes^{7,8}; however, other studies have shown a heterogeneous recovery of naive T lymphocytes during HAART.^{9,10} Thus, the origin of these cells remains controversial. In addition, the number and proportion of circulating naive CD4⁺ T lymphocytes in adults never reaches levels equivalent to those found after immune reconstitution in children.⁴ In total, the abundance of data supports the idea that children have earlier and greater increases in naive CD4⁺ T lymphocytes compared with adults because of a more active thymus, with a greater potential for immune reconstitution when HIV-1 replication is controlled.

T-cell receptor excision circles (TRECs) are the byproduct of T-cell receptor gene rearrangement that are not replicated during mitosis and are diluted during T-cell proliferation. Therefore, TRECs have been used as a marker to monitor recent T-lymphocyte thymic emigrants.^{11,12} In HIV-1–infected adults, quantitation of TRECs has been used to evaluate the thymic function and the status of T-cell immune reconstitution.¹³ TRECs have been also reported to be associated with different clinical markers including age, plasma HIV-1 RNA, CD4⁺ T-lymphocyte counts, CD4⁺ T-lymphocyte percentages, and naive CD4⁺ T-lymphocyte counts in HIV-1–infected children,^{14–17} and TREC levels increase during HAART in HIV-1–infected children.^{14,15} Thus, considerable data support that the level of TREC is a useful marker of thymic function in HIV-1–infected individuals.

In a cohort of children receiving HAART with sustained virologic suppression, we previously reported that those patients whose plasma HIV-1 RNA reached <50 copies/mL after week 8 of treatment (slow responders) had higher HIV-1 intracellular DNA (HIV-1 DNA) than children who achieved plasma HIV-1 RNA < 50 copies/mL within the first 8 weeks of treatment (rapid responders) throughout the 104-week observation period.¹⁸ Despite these differences, slow responders had equal or higher CD4⁺ T-lymphocyte counts than rapid responders. The current research was designed to determine whether the levels of TREC might help to explain the apparent discrepancy between the CD4⁺ T-lymphocyte counts and levels of plasma HIV-1 RNA and HIV-1 DNA observed in the rapid responders compared with slow responders in this cohort of HIV-1–infected children receiving HAART.

METHODS

Subjects

Subjects studied were enrolled in Pediatric AIDS Clinical Trial Group (PACTG) 382, a study designed to evaluate the pharmacokinetics, tolerance, and potential efficacy of efavirenz, nelfinavir, and 1 or more nucleoside analogue reverse transcriptase inhibitors in children.¹⁹

Thirty-one children (median age, 5.6 years; age range, 3.2–16.8 years) were selected for this study on the basis of persistent, undetectable plasma HIV-1 RNA while receiving HAART for >2 years after initiation of study treatment. These children were selected using the following criteria: (1) they had achieved sustained plasma HIV-1 RNA < 50 copies/mL, and (2) they had no more than 2 detectable plasma HIV-1 RNA measurements of >50 copies/mL after reaching RNA < 50 copies/mL. Seventeen (55%) children were ≤5 years old, 9 (29%) children were 6 to 9 years old, and 5 (16%) children were ≥10 years old.

Quantitation of HIV-1 intracellular DNA

HIV-1 DNA levels were quantified by the Amplicor monitor HIV-1 DNA assay (Roche Molecular Systems, Alameda, Calif)²⁰ using previously collected PBMCs at baseline and weeks 2, 4, 8, 20, 48, 80, and 104.¹⁸ The quantities of HIV-1 DNA were expressed as copies per 10⁶ PBMCs as well as per 10⁶ CD4⁺ T lymphocytes. HIV-1 DNA copies per 10⁶ CD4⁺ T lymphocytes was strongly correlated with DNA copies per 10⁶ PBMCs throughout the study period ($r > .85$; $P < .0001$). Thus, DNA expressed as copies per 10⁶ PBMCs was used in the analyses.

Measurement of plasma HIV-1 RNA

The level of plasma HIV-1 RNA was measured by the Amplicor HIV-1 Monitor assay (Roche Molecular Systems). Samples with <400 copies/mL of plasma HIV-1 RNA were retested by using the Ultrasensitive HIV-1 Monitor assay (Roche Molecular Systems, Version 1.0) with a quantitation limit of 50 copies/mL of plasma HIV-1 RNA. The values of the ultrasensitive assay were used for data analysis when samples were retested.

Measurement of CD4⁺ and CD8⁺ T lymphocytes

The absolute numbers and percentages of CD4⁺ and CD8⁺ T lymphocytes were determined in PACTG certified laboratories using flow cytometry at baseline and weeks 2, 4, 8, 20, 48, and 104.

Quantitation of naive and memory T lymphocytes

The quantitation of naive and memory T lymphocytes was performed at a PACTG-Core Immunology laboratory at weeks 20, 48, and 104. CD4⁺ T lymphocytes expressing CD45RA⁺ surface antigen (CD4⁺CD45RA⁺) were considered to be predominantly naive CD4⁺ T-lymphocytes, whereas CD4⁺ T lymphocytes expressing CD45R0⁺ surface antigen were considered to be predominantly memory CD4⁺ T lymphocytes (CD4⁺CD45R0⁺).

Quantitation of TRECs

The TREC fragment was quantified by real-time PCR using specific primers and probes.⁹ PCR reactions were performed in a volume of 20 μL with 400 nmol/L primers (5'-AAA GAG GGC AGC CCT CTC CAA GGC-3' and 5'-AGG CTG ATC TTG TCT GAC ATT TGC TCC-3'), 100 nmol/L probes (5'-AGG GAT GTG GCA TCA CCT TTG TTG ACA-fluorescein isothiocyanate-3', 5'-GGC ACC CCT CTG TTC CCC ACA GGA-LightCycler RED 640-3') (IT Biochem, Salt Lake City, Utah), and 100–400 ng DNA. LightCycler DNA Master Hybridization Probes buffer (Roche Applied Science, Indianapolis, Ind) was used with a final

concentration of Mg^{2+} 3.5 mmol/L. The PCR reaction was as follows: denaturation step at 95°C for 120 seconds, followed by 45 cycles of denaturation (95°C for 0 seconds), annealing (63°C for 40 seconds), and extension (72°C for 40 seconds), followed by melting curve analysis from 45°C to 95°C.

Statistical analysis

Nonparametric tests were used to avoid violating the assumption of normality. For median change in TREC from baseline to each follow-up week, the Wilcoxon matched pairs sign-rank test was used. Correlations among TREC levels and HIV-1 DNA, age, $CD4^{+}$ T lymphocytes, naive $CD4^{+}$ T lymphocytes ($CD4^{+}CD45RA^{+}$), and memory $CD4^{+}$ T lymphocytes ($CD4^{+}CD45RO^{+}$) were calculated by using the Spearman rank-order methods. Multivariate analysis was performed to predict TREC levels using independent covariates such as age, $CD4^{+}$ T-lymphocyte counts, and plasma HIV-1 RNA. All analyses were performed by using the 2-tailed test.

RESULTS

Continuous suppression of plasma HIV-1 RNA is associated with sustained increases in $CD4^{+}$ T-lymphocyte counts and a gradual decline of HIV-1 DNA. All 31 patients had detectable plasma HIV-1 RNA (range, 788–156,417 copies/mL) before initiation of HAART. Sixteen (52%) of 31 patients achieved a plasma HIV-1 RNA level < 50 copies/mL within the first 8 weeks of treatment, and all patients reached an undetectable level by week 48. Plasma HIV-1 RNA remained undetectable in all patients after week 48 through week 104. Median $CD4^{+}$ T-lymphocyte counts significantly increased from baseline (median, 693/ μ L; range, 248–2616/ μ L) to week 20 (median, 928/ μ L; range, 242–2705/ μ L; $P = .001$), extending to week 104 (median, 960/ μ L; range, 260–1921/ μ L; $P = .0001$).

The median levels of HIV-1 DNA were compared at each visit during HAART.¹⁸ The median level of HIV-1 DNA was 750 copies/ 10^6 PBMCs at baseline (range, 204–4464 copies/ 10^6 PBMCs). HIV-1 DNA declined gradually, and statistically significant differences were seen between the median levels of HIV-1 DNA at baseline and at week 8 ($P = .003$) and between the median levels at weeks 20, 48, 80, and 104 ($P < .001$). HIV-1 DNA quantities continued to decrease from week 20 through week 80 ($P = .003$) and reached plateau levels between week 80 and week 104 ($P = .7$) after starting HAART. However, HIV-1 DNA remained detectable in all patients through week 104.

Change in median TREC levels during HAART

Although all subjects had a significant decline in plasma HIV-1 RNA during the early stage of HAART and a gradual increase of $CD4^{+}$ T-lymphocyte counts throughout HAART, interestingly, median TREC levels during HAART did not vary significantly from baseline to each study visit ($P = .08-.83$; $n = 20-30$). The median TREC level was 8721 copies/ 10^5 PBMCs at baseline (interquartile range [IQR], 4029–15,470 copies/ 10^5 PBMCs; $n = 31$). The median TREC levels during HAART were as follows: 8808 copies/ 10^5 PBMCs (IQR, 3130–18,250 copies/ 10^5 PBMCs) at week 8 ($P = .41$), 10,460 copies/ 10^5 PBMCs (IQR, 3331–14,680 copies/ 10^5 PBMCs) at week 48 ($P = .50$), 9499 copies/ 10^5 PBMCs (IQR, 4835–14,205 copies/ 10^5 PBMCs) at week 80 ($P = .08$), and 7975 copies/ 10^5 PBMCs (IQR, 1576–12,400 copies/ 10^5 PBMCs) at week 104 ($P = .64$).

Negative correlation between TREC levels and age

Because age has been reported to be an important determinant for TREC levels in HIV-infected children,^{15,17} the relationship between TREC levels and age was evaluated. Overall, there was

a negative correlation of TREC levels with age ($r = -.21$; $P = .002$), with the strongest inverse correlations at week 8 ($r = -.42$; $P = .02$) and week 80 ($r = -.49$; $P = .03$).

Correlation between TREC levels and virologic markers

Evaluation of TREC levels and HIV-1 DNA revealed a positive correlation between TREC levels and HIV-1 DNA during HAART ($r = .14$; $P = .03$; $n = 225$), most notably at week 48 ($r = .60$; $P = .001$) and week 80 ($r = .61$; $P = .004$), when all patients reached undetectable plasma HIV-1 RNA. However, at week 104, when HIV-1 DNA was at plateau levels, there was not a significant correlation between levels of TREC and HIV-1 DNA ($r = .14$; $P = .48$), likely reflecting a state of equilibrium between virologic control, decreased T-lymphocyte destruction, and the diminished need for new T lymphocytes.

Levels of TREC were next examined in relationship to plasma HIV-1 RNA during the early stage of HAART, when the majority of patients had detectable plasma HIV-1 RNA. No statistically significant differences were seen between TREC levels and plasma HIV-1 RNA at baseline ($r = .16$; $P = .39$), week 2 ($r = .35$; $P = .07$), and week 4 ($r = .34$; $P = .10$). The correlation between the 2 values was not evaluated after week 8 because the majority of patients (52% at week 8, and 87% at week 20) had reached undetectable plasma HIV-1 RNA.

Correlation between TREC levels and immunologic markers

The associations between TREC levels and CD4⁺ T-lymphocyte counts and CD4⁺ T-lymphocyte percentages were evaluated at each study visit. Overall, there was a persistent positive correlation between TREC levels and CD4⁺ T-lymphocyte counts during HAART ($r = .35$; $P < .001$), with statistically significant correlations at week 2 ($r = .46$; $P = .01$) and week 20 ($r = .46$; $P = .03$). Furthermore, TREC levels correlated significantly with CD4⁺ T-lymphocyte percentages during HAART ($r = .35$; $P < .0001$); this was most apparent before and during the early stage of HAART, at baseline ($r = .37$; $P = .04$), week 2 ($r = .56$; $P = .005$), week 4 ($r = .63$; $P < .001$), and week 8 ($r = .47$; $P = .02$).

We next examined the association between TREC levels and subsets of naive and memory T lymphocytes. Data for naive (CD4⁺CD45RA⁺) and memory CD4⁺ T lymphocytes (CD4⁺CD45RO⁺) were available from a subset of patients at weeks 20 ($n = 8$), 48 ($n = 13$), and 104 ($n = 18$). At week 20, there were strong positive correlations between TREC levels and (1) naive CD4⁺ T-lymphocyte counts ($r = .92$; $P < .001$; Fig 1) and (2) naive CD4⁺ T-lymphocyte percentages ($r = .59$; $P = .05$). In contrast, TREC levels were statistically negatively correlated with memory CD4⁺ T-lymphocyte percentages ($r = -.67$; $P = .02$), but not with absolute memory CD4⁺ T-lymphocyte counts ($r = -.37$; $P = .33$). At week 48 and week 104, no significant correlations were seen between TREC levels and naive T-lymphocyte counts ($P = .50$ and $P = .85$, respectively) and naive T-lymphocyte percentages ($P = .50$ and $P = .81$, respectively), suggesting that fewer new thymically derived T lymphocytes were required to maintain adequate levels of naive T lymphocytes.

Rapid responders and slow responders

As noted, subjects were divided into 2 groups on the basis of their responses to HAART: rapid responders ($n = 16$), whose plasma HIV-1 RNA level declined to <50 copies/mL by 8 weeks, and slow responders ($n = 15$), whose plasma HIV-1 RNA level reached <50 copies/mL after 8 weeks. The median week when slow responders reached an undetectable plasma HIV-1 RNA level was week 20. No statistically significant differences were seen at baseline between the rapid responders and slow responders in their baseline characteristics, including sex ($P = .11$), age ($P = .14$), race/ethnicity ($P = .24$), baseline CD4⁺ T-lymphocyte counts ($P = .61$), or baseline plasma HIV-1 RNA ($P = .14$).

Slow responders compared with rapid responders have higher levels of HIV-1 DNA with higher CD4⁺ T-lymphocyte counts throughout HAART. Slow responders had persistently higher median HIV-1 DNA levels throughout HAART compared with rapid responders (Fig 2; $P < .001$). Despite higher median HIV-1 DNA in slow responders, they had equal or higher median CD4⁺ T-lymphocyte counts throughout HAART compared with rapid responders ($P = .12$) that reached significance at week 80 ($P = .05$) and week 104 ($P = .04$; Fig 3).

T-cell receptor excision circle levels in slow responders are consistently higher than levels found in rapid responders. To explain the inconsistency of HIV-1 DNA and CD4⁺ T-lymphocyte counts between rapid responders and slow responders, we hypothesized that TREC levels would be higher in the slow responders compared with rapid responders, considering that slow responders with higher HIV-1 DNA levels will require higher thymic output to maintain high levels of CD4⁺ T lymphocytes. For the entire period of follow-up from baseline through week 104, TREC levels were higher in slow responders than in rapid responders ($P < .001$) and reached significance at week 48 ($P = .004$) and week 80 ($P = .01$; Fig 4).

TREC:HIV-1 intracellular DNA ratios in slow responders and rapid responders

To determine whether there was a consistent and predictable ratio of TREC levels to HIV-1 DNA levels, TREC:HIV-1 DNA ratios were compared between slow responders and rapid responders at each study visit. No significant differences of TREC:HIV-1 DNA ratio were seen between slow responders and rapid responders at any study visit during HAART ($P > .34$).

DISCUSSION

The goal of this study was to examine in a cohort of children the apparent inconsistency between CD4⁺ T-lymphocyte counts and HIV-1 DNA. Our findings show that the group categorized as slow responders had consistently higher TREC levels compared with rapid responders throughout HAART. Thus, despite the presence of persistently higher quantities of HIV-1 DNA, the slow responder group was able to sustain equivalent to higher CD4⁺ T-lymphocyte counts by maintaining a higher release of new thymic emigrants into the circulation.

T-cell receptor excision circle levels were also associated with CD4⁺ T-lymphocyte percentages and counts, suggesting that TREC may be a useful marker to determine the ability of children receiving HAART to sustain T-lymphocyte levels. Our findings suggest that at an early stage of HAART, TREC levels represent active thymopoiesis that serves to replenish CD4⁺ T lymphocytes that were lost through HIV infection as well as normal T-cell turnover. With sustained suppression of plasma HIV-1 RNA and stable CD4⁺ T-lymphocyte counts, the correlation of TREC with HIV-1 DNA remains, suggesting that a low level of intracellular viral replication continues even when patients have normal CD4⁺ T-lymphocyte counts with sustained virologic suppression.^{21,22} The effect of such low-level replication on subsequent immune reconstitution remains to be elucidated.

T-cell receptor excision circle levels have been reported to increase during HAART in HIV-1-infected children^{14,15}; however, in our study, TREC levels did not change significantly. It is likely that the difference in our study compared with previous cohorts is a result of the fact that we selected a cohort of subjects who responded well to HAART and maintained sustained plasma HIV-1 RNA < 50 copies/mL. Under these conditions, the thymic output necessary to sustain a child's T-lymphocyte set point likely approximates that of uninfected children with fewer T lymphocytes needing to be replaced. In addition, the children studied in our cohort appear to have had good thymic reserve, as indicated by their ability to produce adequate TREC levels even before initiating potent anti-retroviral treatment.

In agreement with previous studies in children and adults,^{17,23,24} our data demonstrate that there is a correlation between TREC levels and naive CD4⁺ T lymphocytes. These findings suggest that ongoing production of naive CD4⁺ T lymphocytes in the thymus is most active during the earlier stages of HAART and declines later during HAART therapy. However, the precise timing of when children show maximum thymic output during HAART will require further investigation and may be dependent on the relative potency of specific treatment regimens in suppressing viral replication, the amount of thymic reserve, and the CD4⁺ T-lymphocyte count at the time of initiating treatment.

We appreciate that there are some limitations of the current study. First, TREC levels were quantified in PBMCs rather than in isolated CD4⁺ T lymphocytes, which could potentially affect the levels of TREC. It should be noted, however, that in a previous report in HIV-1–infected children, TREC levels determined in PBMCs correlated well with the TREC levels identified from isolated CD4⁺ T lymphocytes.¹⁵ In our current study, we were not able to examine peripheral T-lymphocyte division in secondary lymphoid organs that could also affect TREC levels.²⁵ Therefore, it is possible that then expression of T lymphocytes could dilute the TREC levels in our assay. However, the consistency of the TREC levels, found in individual patients over time and overall patient groups, would suggest that lymphocyte dilution is unlikely to have altered our findings. In addition, our strong correlation between TREC levels and naive CD4⁺ T lymphocytes ($r = .92$; $P < .001$) further supports the validity of our findings.

Several additional factors have been reported to affect immune reconstitution in HIV-1–infected persons. Growth hormone or growth hormone induction of insulin-like growth factor has been implicated in lymphocyte development and function.²⁶ Because the production of these hormones is elevated during infancy and young childhood, this could greatly contribute to the thymic function. Also, IL-7 has been shown to increase thymic function and plays an important role in thymopoiesis in HIV-1–infected adults and children receiving HAART.^{27–29} Further studies are necessary to investigate the role of these potential contributing factors that affect thymic output and immune reconstitution in HIV-1–infected children.

In conclusion, in children with sustained, undetectable plasma HIV-1 RNA during HAART, our results indicate that TREC levels are strongly associated with HIV-1 intracellular DNA and CD4⁺ T-lymphocyte counts/percentages at different stages of HAART. The ability of HIV-1–infected children receiving HAART to maintain CD4⁺ T-lymphocyte counts with a high HIV-1 intracellular DNA burden is associated with maintenance of TREC levels. Thus, it appears that a robust process enables the thymus to promote immune function by producing new CD4⁺ T lymphocytes in the presence of persistent intra-cellular virus.

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Abbreviations

HAART

Highly active antiretroviral therapy

HIV-1 DNA

HIV-1 intracellular DNA

IQR

Interquartile range

PACTG

Pediatric AIDS Clinical Trial Group

TREC

T-cell receptor excision circle

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FIG 1.

Positive correlation between TREC levels and naive CD4⁺ T-lymphocyte counts. There was a strong positive correlation between TREC levels and naive CD4⁺ T-lymphocyte counts (CD4⁺CD45RA⁺; $r = .92$; $P < .001$) at week 20.

FIG 2.

Comparison of median HIV-1 intracellular DNA levels between rapid responders (HIV-1 RNA < 50 copies/mL by week 8; *white bars*) and slow responders (HIV-1 RNA < 50 copies/mL after week 8; *gray bars*). Slow responders had persistently higher median HIV-1 intracellular DNA levels compared with rapid responders during HAART ($P < .001$). Data shown are expressed as the median value $\pm$ 25th and 75th percentiles. * $P < .05$; ** $P < .01$.

FIG 3.

Comparison of CD4⁺ T-lymphocyte counts between rapid responders (HIV-1 RNA < 50 copies/mL by week 8; *white bars*) and slow responders (HIV-1 RNA < 50 copies/mL after week 8; *gray bars*). Slow responders had equal or higher median CD4⁺ T-lymphocyte counts throughout HAART compared with rapid responders ($P = .12$) that reached significance at week 80 ($P = .05$) and week 104 ($P = .04$). Data shown are expressed as the median value $\pm$ 25th and 75th percentiles. * $P < .05$.

FIG 4.

Comparison of TREC levels between rapid responders (HIV-1 RNA < 50 copies/mL by week 8; *white bars*) and slow responders (HIV-1 RNA levels < 50 copies/mL after week 8; *gray bars*). TREC levels were higher in slow responders than in rapid responders during HAART ($P < .001$) and reached significance at week 48 ($P = .004$) and week 80 ($P = .01$). Data shown are expressed as the median value $\pm$ 25th and 75th percentiles. * $P < .05$.