

## Increased Thymic Output after Initiation of Antiretroviral Therapy in Human Immunodeficiency Virus Type 1–Infected Children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 Trial

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To investigate the thymic contribution to immune reconstitution during antiretroviral therapy (ART), T cell receptor gene rearrangement excision circles (TRECs) were measured in peripheral blood mononuclear cells (PBMC) and CD4 cells from 33 human immunodeficiency virus (HIV) type 1–infected children monitored for 96 weeks after ART initiation. Baseline TREC levels were associated positively with baseline CD4 cell percentage and inversely with age and HIV-1 RNA load. During therapy, TREC level changes in PBMC and CD4 cells were fairly comparable. TREC level changes were inversely related to baseline CD4 cell percentage and positively associated with CD4 cell percentage increases, the main source being naive CD4 cells. TREC changes were independent of age and baseline HIV-1 RNA load; however, HIV-1 suppression was independently associated with smaller TREC changes. Thymic output appears to be the main source of CD4 cell repopulation in children receiving ART. Recovery of thymic function is independent of age and influenced by the status of peripheral CD4 cell depletion and HIV-1 suppression.

Treatment with antiretroviral therapy (ART) reduces mortality and progression of human immunodeficiency virus (HIV) type 1 disease in adults and children [1–3]. Although response to ART in terms of virus suppression is broadly similar in adults and in children, several studies have outlined differences in the pattern of peripheral immune reconstitution. In adults, the peripheral CD4 cell repopulation is a biphasic process with an

initial rapid increase in memory CD4 cells followed by a slower and smaller increase in naive CD4 cells; however, in children, repopulation occurs mainly with naive CD4 cells, and there is only a small increase in memory CD4 cells [4–6]. The substantial increase in naive CD4 cells could be due to expansion and/or prolongation of survival of peripheral CD4 naive cells or increases in thymic output. Given the involution of thymus with age, these differences might be explained by the greater regenerative capacity of the thymus in children.

During intrathymic T cell differentiation, progenitor cells undergo rearrangement of the T cell receptor, resulting in the formation of episomal DNA by-products, termed T cell receptor rearrangement excision circles (TRECs). Since TRECs do not replicate with mitosis and are thus diluted with cellular division, their detection in peripheral blood cells has been proposed as a marker to evaluate the thymopoietic capacity [7].

TREC measurements have been used to evaluate thymic output in several studies of HIV-1–infected children, and an increase in TREC levels was found consistently after ART initiation [8–10]. However, most studies are either cross-sectional or have limited longitudinal follow-up, so that evaluation of the factors that contribute to changes in TREC levels during ART remains limited. In this study, we considered TREC levels in previously untreated children participating in a randomized trial of children receiving combinations of zidovudine (ZDV), lamivudine (3TC), abacavir (ABC), and nelfinavir (NFV) [11]. We investigated the effects on TREC response of age, T cell subsets, and plasma HIV-1 RNA loads at baseline and during ART.

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Ethics committees at all participating centers approved the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial protocol and the taking of samples for further immunological testing. All primary care givers gave written consent for study participation; additional written consent was obtained from children, when appropriate, according to their age and knowledge of human immunodeficiency virus status.

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No member of the writing committee has commercial or other associations that might pose a conflict of interest.

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## Subjects and Methods

**Subjects and samples.** For 33 of 128 children (18 from Italy and 15 from the United Kingdom) participating in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial [11], sequential cellular samples were available for TREC analysis in peripheral blood mononuclear cells (PBMC) at baseline and at 4, 12, 24, 48, and 96 weeks after initiation of ART (10 clinical centers). TREC levels were also measured at the same time points in purified CD4 cells in 12 of 33 children with adequate samples available. CD4 CD45RA (naive)/RO (memory) and CD8 CD45RA/RO immunophenotyping was performed in real time for a subset of 17 of these children plus another 9 children not included in the TREC substudy (26 children total: 3 from Germany, 6 from Italy, and 17 from the United Kingdom). CD4 and CD8 CD45RA and CD45RO were evaluated at baseline, 2 weeks, every 4 weeks from weeks 4 to 24, every 8 weeks to week 72, and every 12 weeks thereafter and are expressed as a percentage of total lymphocytes.

**Laboratory methods.** As part of the main trial, T cell lymphocyte subsets were measured by flow cytometry at each clinical center. Plasma HIV-1 RNA load was measured at a central laboratory (Covance Central Laboratory Services, Geneva) by the Roche Amplicor ultrasensitive assay (version 1.5; limit of detection, 50 HIV-1 RNA copies/mL). Any specimen showing >40,000 HIV-1 RNA copies/mL on the ultrasensitive assay was retested by standard assay (limit of detection, 400 HIV-1 RNA copies/mL).

**Isolation of CD4 cells and TREC quantification.** CD4 cells were isolated from PBMC by using magnetic beads coated with monoclonal antibodies against human CD4 (Dynabeads M-5450; Dynal), according to the manufacturer's instructions. TREC levels were analyzed by real-time quantitative polymerase chain reaction (PCR) assay [10]. Cells were lysed as reported elsewhere [12]. For PCR amplification template, we used 10  $\mu$ L of cell lysate, equivalent to 80,000 cells. The reaction volume was 50  $\mu$ L, which contained, in addition to the template, 1.0 $\times$  TaqMan Universal PCR Master Mix (PE Applied Biosystems), 300 nM each primer (forward, 5'-CACATCCCTTTCAACCATGCT-3'; reverse, 5'-GCCA-GCTGCAGGGTTTAGG-3'), and 100 nM fluorogenic probe (5'-ACACCTCTGGTTTTGTAAAGGTGCCACT-3') conjugated with the fluorophores FAM (6-carboxyfluorescein) at the 5'-end and TAMRA (6-carboxytetramethylrhodamine) at the 3'-end.

The PCR primers and the fluorogenic probe were specifically designed for the detection of human TRECs. The thermal cycling conditions were 2 min at 50°C, 10 min at 95°C, and 45 cycles each at 95°C for 15 s and 60°C for 1 min. The reaction was performed in a spectrofluorometric thermal cycler (ABI PRISM 7700 Sequence Detector; PE Applied Biosystems). For each run, a standard curve was generated from duplicate samples of 5-fold serially diluted known copies of plasmid DNA obtained by inserting a human signal joint TREC fragment in the pCR-Blunt Vector (Invitrogen; Life Technologies). Under our experimental conditions, the assay was sensitive enough to detect 2.5 copies of TRECs and showed a dynamic range of  $\geq 5 \log_{10}$  (from 2.5 to  $2 \times 10^5$  copies) [10]. Each sample was run in duplicate; a threshold cycle (Ct) value for each duplicate was calculated by determining the point at which the fluorescence exceeded the threshold limit. We used the mean Ct value of the 2 duplicates plotted against the standard curve to calculate the TREC number in the sample. The mean coefficient

of variation of intrasample Ct values was 0.4% (median, 0.3%; range, 0.02%–0.9%).

To normalize for cell equivalents, the  $\beta$ -actin gene was quantified under real-time PCR conditions similar to those used for TREC quantification. The primer and probe concentrations were as follows: 300 nM forward primer (5'-TCACCCACACTGTGCCCATCTACGA-3'), 600 nM reverse primer (5'-CAGCGGAACCGCTCATTGCCAATGG-3'), and 200 nM fluorogenic probe (5'-ATGCCCTCCC-CATGCCATCCTGCGT-3') conjugated with the fluorophore VIC (PE Applied Biosystems) at the 5'-end, and TAMRA at the 5'-end. The  $\beta$ -actin standard curve was obtained from 5-fold serial dilutions of DNA extracted from the 8E51 cell line. Results were expressed as TREC copies/ $10^6$  cells ( $2 \times 10^6$   $\beta$ -actin copies).

**Statistical methods.** The closest value within equally spaced windows between scheduled assessment weeks was used to calculate changes from baseline. Plasma HIV-1 RNA loads and TREC results were  $\log_{10}$  transformed.  $\log_{10}$  TREC levels in PBMC and CD4 cells were compared using Spearman's  $\rho$ . Mean changes in  $\log_{10}$  TREC levels from baseline were compared by use of pointwise *t* tests, with generalized estimating equations providing a global test of a difference from baseline across correlated results from weeks 4 to 96 [13].

Because there were limited data on TREC levels in sequential CD4 cellular samples, investigation of the factors associated with change in TREC level was restricted to TRECs measured in PBMC. Statistical analysis of whether the change in  $\log_{10}$  TREC level depends on its initial value is complicated by the problems of regression to the mean and errors in the measurement of the initial baseline value [14]. Therefore, to correctly estimate the relationship between change in TREC level and initial value, the absolute  $\log_{10}$  TREC values at 0, 4, 12, 24, 48, and 96 weeks were jointly regressed on assessment week (categorical) and other factors by using multi-level models [15], with random effects for the baseline value and each assessment week to account for correlation over time (fitting a common effect at weeks 4 and 12). The association between baseline TREC level and subsequent changes is therefore modeled through these correlation parameters, so tests of statistical significance have low power to detect genuine relationships. A direct assessment of the effect of the baseline on subsequent TREC levels was estimated by comparing conditional population means of the predicted TREC levels.

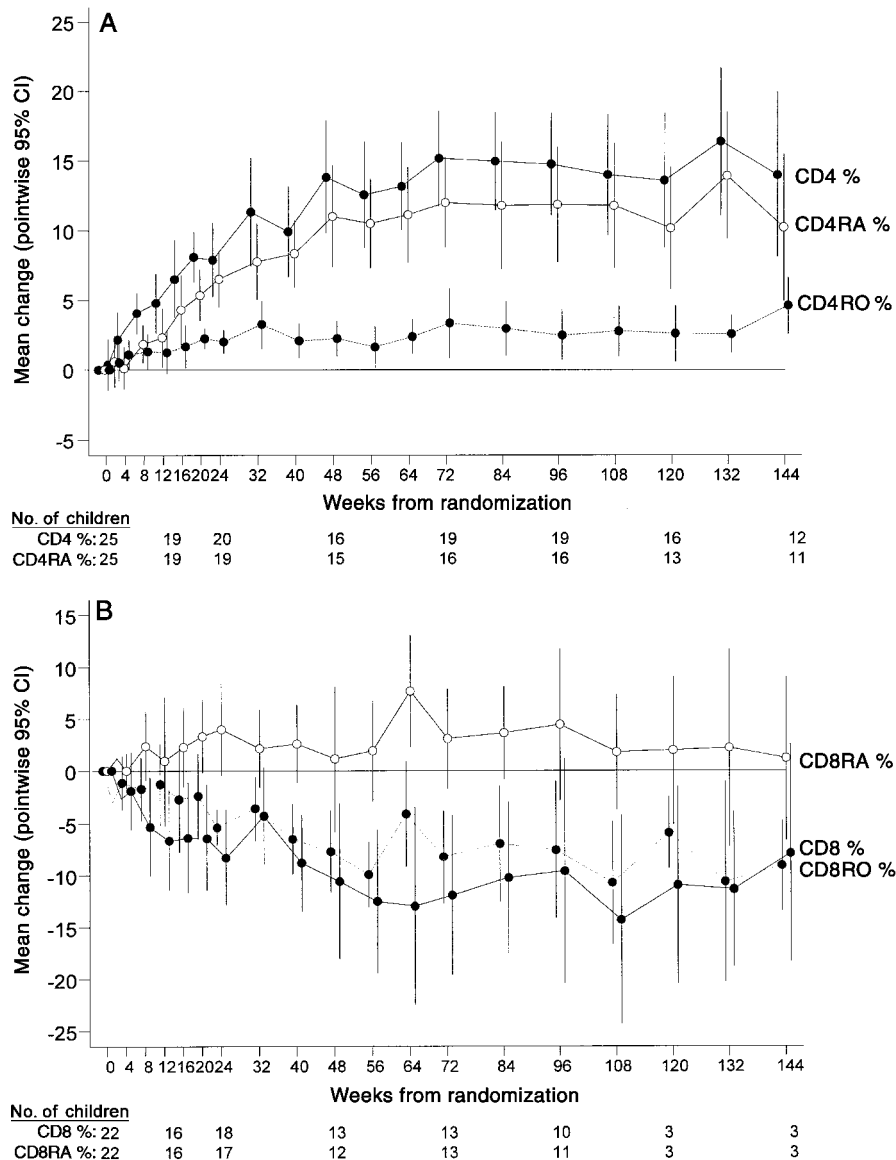
Baseline CD4 cell percentage,  $\log_{10}$  HIV-1 RNA load, and age were included in all models as predictors of the true baseline TREC level. The additional effect on changes in TREC level at subsequent assessment weeks of these baseline factors, as well as changes in CD4 cell percentage and  $\log_{10}$  HIV-1 RNA load, were then investigated. The form of any effect found was also considered by use of natural cubic splines [16] and predefined categorization (e.g.,  $\log_{10}$  HIV-1 RNA load <50 or 400 copies/mL). The effects of CD4 and CD8 isophenotypes at baseline and changes at subsequent assessment weeks on changes in TREC level were also explored in children for whom these values were available. Having identified significant predictors of changes in  $\log_{10}$  TREC level, Wald tests of heterogeneity were used to assess whether the effect of these predictors varied across assessment weeks.

**Results**

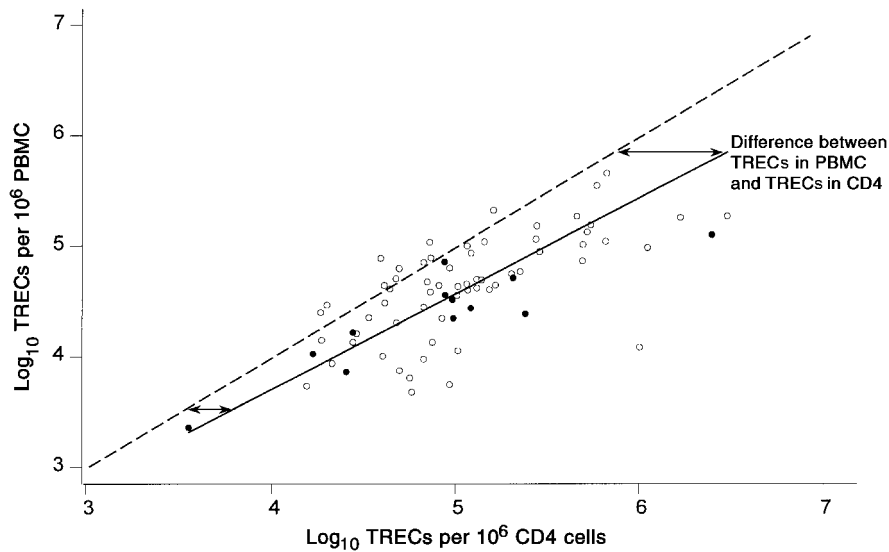
*Population.* At baseline, the median age of the 33 ART-naive children was 7.1 years (range, 0.3–15.5 years). Four children (12%) had AIDS at randomization. Children were randomly assigned to receive ZDV plus 3TC ( $n = 8$ ), ZDV plus ABC ( $n = 13$ ), or 3TC plus ABC ( $n = 12$ ). Sixteen children with more advanced disease also received NFV; 17 with asymptomatic disease received NFV alone ( $n = 10$ ) or placebo matched to NFV ( $n = 7$ ) in a second randomization. None of the 7 children receiving only double nucleoside reverse-transcriptase inhibitors changed from randomized dual therapy during the course of the trial, and all but 1 had HIV-1 RNA

loads decline to <400 copies/mL. Overall, these children took  $\geq 2$  antiretrovirals for >99% of the study time and received triple therapy for 73% of the study time.

*CD4 cells and plasma HIV-1 RNA.* At baseline, the median CD4 cell percentage was 17% (interquartile range, 9%–24%), and the mean plasma HIV-1 RNA load was 5.01 log<sub>10</sub> copies/mL (SD, 0.76 log<sub>10</sub> copies/mL), compared with 22% and 5.02 log<sub>10</sub> copies/mL in the trial overall [11]. Virus suppression in the 33 children generally reflected the overall trial results [11]. At 48 weeks after therapy initiation, 50% had HIV-1 RNA loads <400 copies/mL (43% had <50 HIV-1 RNA copies/mL), and the overall mean decrease in HIV-1 RNA load was 2.34 log<sub>10</sub>



**Figure 1.** Changes in CD4 and CD8 phenotypes after initiation of antiretroviral therapy. *A*, CD4, CD4RA (naive), and CD4RO (memory) cells as percentages of total lymphocytes. *B*, CD8, CD8RA, and CD8RO cells as percentages of total lymphocytes. CI, confidence interval.



**Figure 2.** T cell receptor gene rearrangement excision circles (TRECs) in peripheral blood mononuclear cells (PBMC) and CD4 cells. Of note, data (solid line shows fit) must overlay dashed line for perfect concordance. Horizontal distance between dashed and solid lines represents over-/underestimation. ●, Samples taken 2–8 weeks after randomization; ○, samples taken before or >8 weeks after randomization.

copies/mL (SD, 1.23 log<sub>10</sub> copies/mL). Among the children for whom immunophenotyping was done (*n* = 26), the mean change from baseline log<sub>10</sub> HIV-1 RNA remained stable at about a 3 log<sub>10</sub> drop beyond 144 weeks, with estimated mean log<sub>10</sub> HIV-1 RNA load of 50–400 copies/mL from week 8 to beyond week 144. Only 2 children with immunophenotyping data never achieved HIV-1 RNA suppression to <400 copies/mL at any time during follow-up.

**Immunophenotypes.** The early increase in CD4 CD45RA percentage in the PENTA 5 trial described to 1 year after ART initiation [4] was sustained beyond 2 years (figure 1). The mean increase in CD4 cell percentage plateaued at ~15% after 48 weeks (12% increase in CD45RA and 3% increase in CD45RO; both *P* = .0001, over all follow-up). Beyond 64 weeks, values had normalized to a mean CD4 cell percentage of ~30% (CD45RA, 22%; CD45RO, 8%). In contrast, the mean change in CD8 cell percentage plateaued at about an 8% decrease at week 48, with a 10% loss in CD8 CD45RO cells and a 2% increase in CD8 CD45RA cells (*P* < .0001 for both, over all follow-up; figure 1).

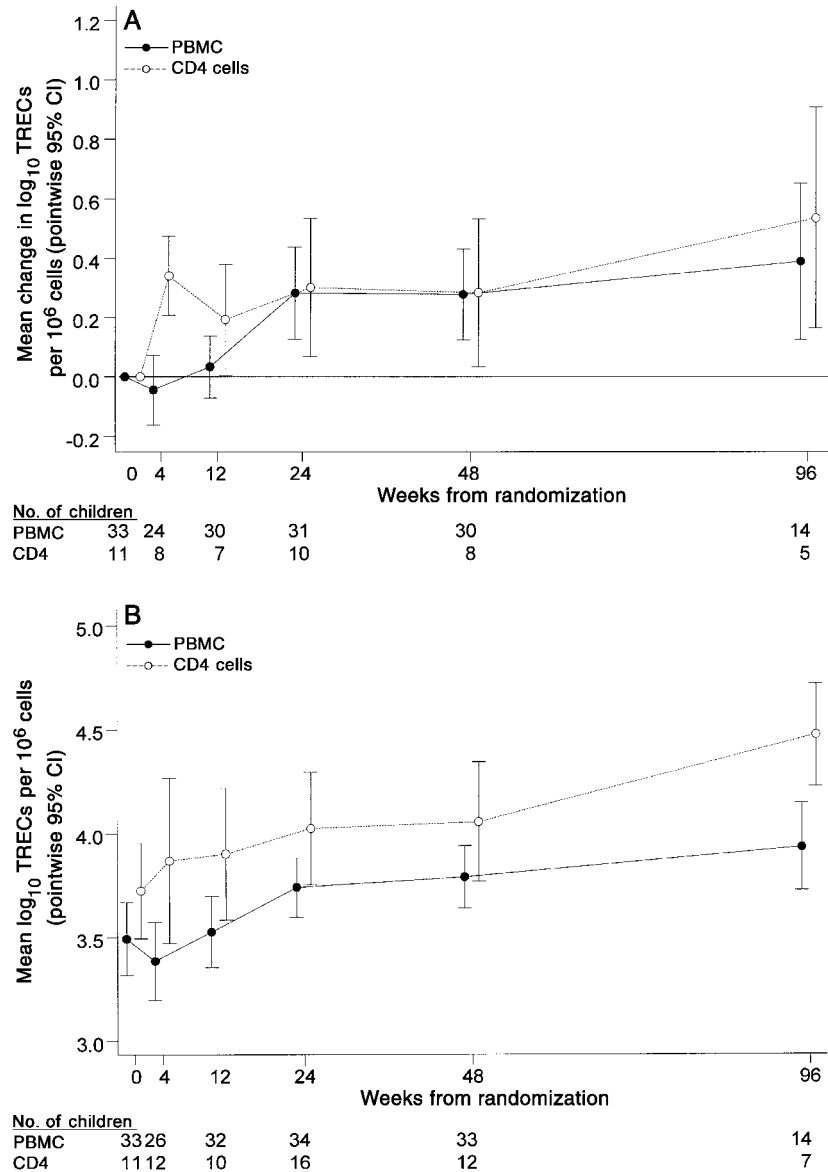
**TREC levels in PBMC and CD4 cells.** For 74 samples, there were TREC results for both PBMC and CD4 cells. As expected, TREC levels in PBMC were lower than in CD4 cells (figure 2) with an average of 0.42 excess log<sub>10</sub> TRECs in CD4 cells. However, the overall agreement between log<sub>10</sub> TREC levels in PBMC and CD4 cells was high (Spearman's  $\rho$ , 0.70; *P* < .0001). There was a suggestion that the underestimation of TREC levels in PBMC, compared with those in CD4 cells, marginally increased as log<sub>10</sub> TREC levels increased, but this was not statistically significant (*P* = .09).

There was a significant increase in log<sub>10</sub> TREC levels in both

PBMC and CD4 cells after baseline (*P* < .0001 for both; figure 3). The mean increase in log<sub>10</sub> TRECs/10<sup>6</sup> PBMC and CD4 cells at week 48 was 0.28 log<sub>10</sub> (95% confidence interval [CI], 0.12–0.44 log<sub>10</sub>; *P* = .001, *t* test) and 0.28 log<sub>10</sub> (95% CI, –0.02 to 0.59 log<sub>10</sub>; *P* = .06, *t* test), respectively. During therapy, the change in TREC level appeared to be similar in PBMC and CD4 cells, with the exception of changes at week 4 after the initiation of ART (figure 3). There was no evidence that the relative difference between TREC levels in CD4 cells and PBMC was larger at this stage, compared with overall findings (figure 2).

**Effect of age, CD4 cells, and HIV-1 RNA load on TREC levels at baseline.** At baseline, the median TREC level was 37,090 TRECs/10<sup>6</sup> PBMC (range, 1260–167,910 TRECs/10<sup>6</sup> PBMC). In a univariate analysis, TREC levels in PBMC at baseline were strongly related to baseline CD4 cell percentage and inversely related to age at baseline (figure 4). When we included these factors and baseline HIV-1 RNA load in a joint multilevel model, baseline log<sub>10</sub> TREC level was independently and inversely related to baseline log<sub>10</sub> HIV-1 RNA load (*P* = .008), positively related to baseline CD4 cell percentage (*P* = .002), and inversely related to age at baseline (*P* < .0001). There was no independent effect of AIDS status at baseline on baseline TREC level (*P* = .83).

**Effect of baseline factors on TREC level changes during ART.** Children with higher baseline TREC levels tended to have smaller increases in subsequent TREC values, although this was not statistically significant (*P* = .46). Thus, a 1 log<sub>10</sub> higher TREC level at baseline was estimated to be independently associated with, on average, a 0.10, 0.64, 0.34, and 0.47 smaller



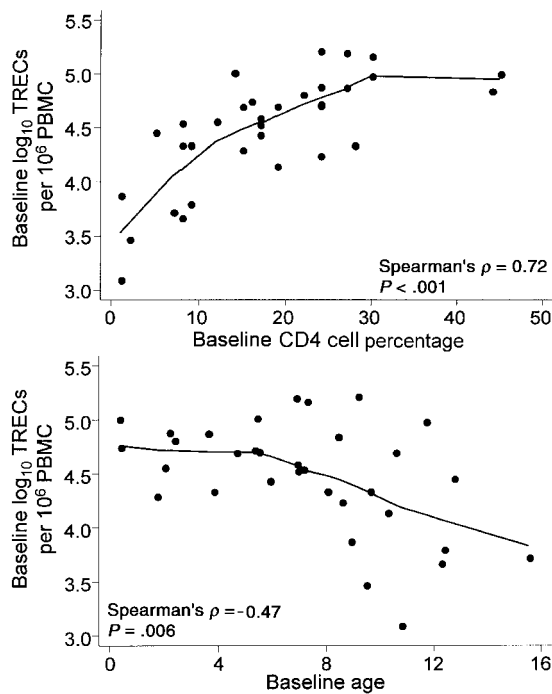
**Figure 3.**  $\log_{10}$  T cell receptor gene rearrangement excision circles (TRECs) after initiation of antiretroviral therapy. *A*, Mean change in  $\log_{10}$  TRECs/ $10^6$  cells from randomization. *B*, Mean absolute  $\log_{10}$  TRECs/ $10^6$  cells from randomization. CI, confidence interval; PBMC, peripheral blood mononuclear cells.

change in  $\log_{10}$  TREC level at weeks 4 and 12, 24, 48, and 96, respectively.

With regard to other factors, although age at baseline and baseline  $\log_{10}$  HIV-1 RNA loads were strongly associated with the baseline TREC level, neither age ( $P = .65$ ) nor baseline  $\log_{10}$  HIV-1 RNA load ( $P = .32$ ) was associated with subsequent increases in  $\log_{10}$  TREC level, nor was there any effect of AIDS status at baseline ( $P = .27$ ). The only baseline factor found to be a significant independent predictor of subsequent changes in  $\log_{10}$  TREC level was baseline CD4 cell percentage.

A higher baseline CD4 cell percentage was associated with a smaller change in  $\log_{10}$  TREC level subsequently (on average, 0.04 smaller change in  $\log_{10}$  TREC level for each 5% higher baseline CD4 cell percentage; 95% CI, 0.00–0.07;  $P = .05$ ). This effect increased over time with a 5% higher CD4 cell percentage at baseline predicting a 0.00, 0.04, 0.08, 0.13, and 0.15 smaller change in  $\log_{10}$  TREC level at weeks 4, 12, 24, 48, and 96, respectively ( $P = .001$ , for heterogeneity; figure 5).

*Effect of baseline factors and subsequent changes in CD4 cell percentage and HIV-1 RNA load on TREC level changes during*



**Figure 4.** Univariate relationship between T cell receptor gene rearrangement excision circles (TRECs) in peripheral blood mononuclear cells (PBMC) at baseline and baseline CD4 cell percentage and age (years). Data were smoothed using the Lowess method (bandwidth, 0.95).

**ART.** Larger changes in CD4 cell percentage after baseline were associated with larger changes in log<sub>10</sub> TREC level (on average, 0.08 larger change in log<sub>10</sub> TREC level for each 5% larger change in CD4 cell percentage; 95% CI, 0.04–0.13; *P* = .0006). HIV-1 RNA suppression to <50 copies/mL was also inversely related to TREC change, with a 0.12 smaller change in log<sub>10</sub> TREC (95% CI, 0.01–0.24; *P* = .04) when HIV-1 RNA load was <50 copies/mL after adjusting for change in CD4 cell percentage. However, there was no evidence of an additional effect of absolute log<sub>10</sub> HIV-1 RNA load or change in log<sub>10</sub> HIV-1 RNA load at each assessment week on change in log<sub>10</sub> TREC level (*P* = .28 and *P* = .16, respectively).

**CD4 phenotype and TREC changes during ART.** TREC level information was available for 17 children with immunophenotyping data. After adjusting for HIV-1 RNA suppression to <50 copies/mL, as described above, change in CD45RA cell percentage (of total lymphocytes) predicted changes in TREC level similar to total CD4 cell percentage (on average, 0.13 larger change in log<sub>10</sub> TREC level for each 5% larger change in CD45RA cell percentage; 95% CI, 0.06–0.20; *P* = .0004). There was no significant effect of change in CD45RO cell percentage (on average, 0.04 smaller change in log<sub>10</sub> TREC level for each 5% larger change in CD45RO percentage; 95% CI, 0.16 smaller to 0.08 larger change; *P* = .52).

**Discussion**

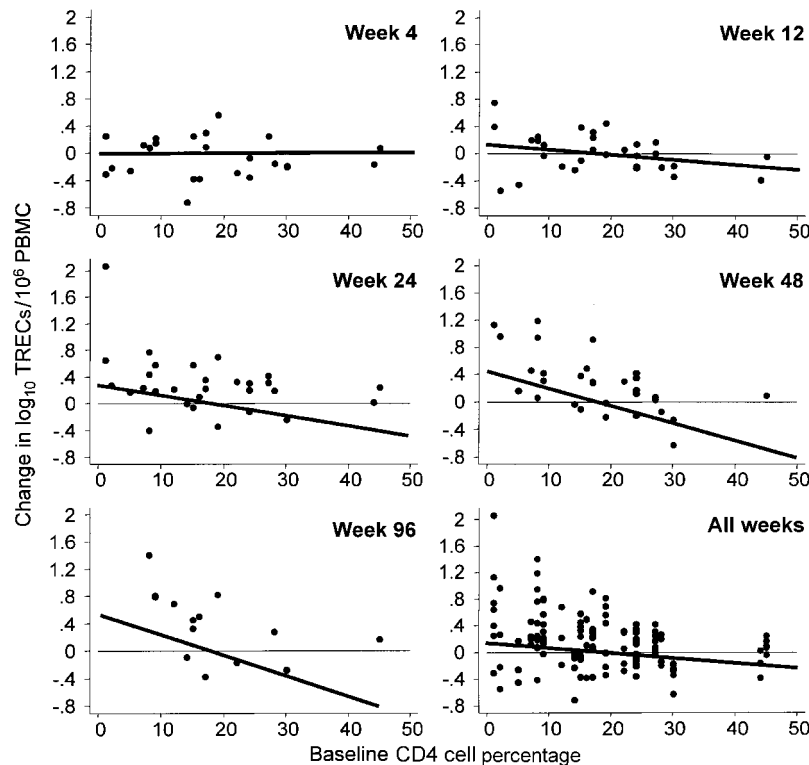
We reported elsewhere that the increase in CD4 cells after ART in children was predominantly due to naive CD4 CD45RA cells [4]. The source of these cells was not established, but a recent study presents evidence that the increase in naive CD4 CD45RA cells is likely thymically derived [8]. However, this study focused on univariate analyses and, in addition, included both previously treated and untreated children and a mixture of cross-sectional and longitudinal data.

In the current study, we used a multivariate model to examine factors associated with change in TREC levels over time. This model took into account baseline age, CD4 cell percentage, and HIV-1 RNA load, plus changes in CD4 cell percentage and HIV-1 RNA load during therapy. This is important because age, CD4 cell percentage, and HIV-1 RNA load are all highly confounded, and it is impossible to disentangle the independent effects of these variables by using only univariate analyses. Furthermore, in this study, all children were previously untreated, and samples were tested at frequent fixed time points after initiation of ART.

In agreement with previous studies [8, 10], we found that TREC values in PBMC were highly correlated with those in CD4 cells. Because CD4 cells represent a TREC-enriched fraction, compared with total PBMC, the absolute TREC numbers would be expected to be lower in PBMC than in CD4 cells; however, TREC changes in PBMC and in CD4 cells were fairly comparable, with the exception of shortly after initiation of therapy when TREC increases were observed in CD4 cells but not in PBMC. It is possible that an increase in TREC-positive cells, noticeable in the CD4 cell fraction, was masked in the total PBMC sample at this time point because of a dilution effect, possibly due to early increases in other cells, including B cells [17].

At baseline, we observed a positive correlation between TREC levels in PBMC and CD4 cell percentage and an inverse association with age, as reported elsewhere [8, 10]. In addition, in the multivariate analysis, we observed that higher baseline TREC levels were independently associated with a lower HIV-1 RNA load at baseline. This additional effect of baseline HIV-1 RNA load might reflect a peripheral dilution of cells containing TREC due to increased cell division resulting from the persistent activation of the immune system by HIV-1 [18].

We found that change in TREC level after ART was inversely related to baseline CD4 cells. Restoration of the thymic function was therefore strongly influenced by the status of peripheral CD4 cell depletion at therapy initiation, consistent with the concept that the thymus plays a key role in T cell homeostasis—the higher the T cell depletion in the periphery, the greater the output. Of note, this inverse relationship between TREC changes and baseline CD4 cell percentage was less evident during the first weeks of therapy when most of the children still had high, albeit falling, HIV-1 RNA loads, but emerged with increasing weeks of therapy



**Figure 5.** Variation in the effect of baseline CD4 cell percentage on subsequent change in T cell receptor gene rearrangement excision circles (TRECs) in peripheral blood mononuclear cells (PBMC) at different assessment weeks. *Solid lines*, estimated effect of baseline CD4 cell percentage on subsequent changes in  $\log_{10}$  TREC level, as estimated in a multilevel model that included only baseline factors. Estimated effects shown at individual assessment weeks and averaged over all assessment weeks.

and HIV-1 suppression. There was also an apparent inverse relationship between change in TREC level following ART and baseline TREC level. However, the statistical test for such an association had very low power with only 33 children and it was not conventionally statistically significant.

In contrast to previous studies [8], we observed that age at initiation of therapy did not influence changes in TREC level following ART, suggesting that starting ART at any time throughout childhood can result in high thymic output. This is in agreement with recent data showing that normalization of CD4 cell counts in children treated with highly active ART is independent of age [19]. Only 4 children in our study were >12 years old, so we cannot comment on effects during adolescence. Of note, in HIV-1-uninfected children, there is a sharp decline in TREC level during the first 10–15 years of life. However, this is mainly due to a decrease in total lymphocytes during this period rather than to a decline in the percentage of TREC-positive lymphocytes [20].

CD4 cell percentage increases during therapy were strongly associated with increases in TREC level. In addition, we found that children with HIV-1 RNA loads <50 copies/mL had smaller increases in  $\log_{10}$  TREC levels, independent of their immuno-

logic response, although no correlation between change in TREC level and either baseline HIV-1 RNA load or change in HIV-1 RNA load during ART was observed.

Several children in this study had a discordant virologic and immunologic response to therapy. For example, at 48 weeks, 7 of 30 had >10% increases in CD4 cells despite a lack of HIV-1 suppression to <50 copies/mL. Such a discordant response to therapy in children [8, 10] and adults [21, 22] was reported elsewhere. In agreement with previous observations [8, 10], an increase in TREC level was observed in all of the children studied. The finding that HIV-1 RNA suppression is associated with a lower thymic output supports the concept that, unless full virus suppression occurs, higher thymic output is required to compensate for greater peripheral CD4 cell depletion in children with detectable circulating virus, compared with those achieving full suppression. The mechanisms that underlie the recovery of thymic function in sustaining the T cell homeostasis, despite the persistence of HIV-1, remain to be clarified.

Our immunophenotyping data showed that naive CD4 CD45RA cells contributed to most of the CD4 cell increase and that these increases were sustained for >2 years. A smaller increase in memory CD4 CD45RO cells, which may result from

both the expansion of peripheral memory cells and the cellular switching from a naive to a memory phenotype, was also observed. Our data provide some indication of the mechanisms by which T cell repopulation occurs after ART. Because the increase in naive CD4 CD45RA cells, but not memory CD4 CD45RO cells, was closely related to TREC changes, an increase in replication of naive cells in the periphery is unlikely because this would not be associated with increased TREC levels. Both longer survival of naive cells and increased thymic output could explain our results, and we suggest that the relative contributions of each of these may be different in children with full virologic suppression, compared with those with continuing viral replication. Thus, in children with HIV-1 RNA loads <50 copies/mL, naive cells may survive longer, obviating the need for such a large output of new cells from the thymus.

A number of key questions remain unanswered in the treatment of HIV-1-infected children. In particular, the optimal timing of ART initiation has yet to be established. This is the most extensive longitudinal study to date examining the relationship between ART and TREC level in previously untreated children. Our results indicate that, even in older children with low CD4 cell counts and high virus loads, there is evidence of significant capacity for thymic production of CD4 cells. Although the clinical significance of these findings may not become apparent for some time, the degree of immunosuppression and age at ART initiation may not necessarily adversely affect the capacity for immunologic regeneration.

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