Changes in T Cell Receptor Excision DNA Circle (TREC) Levels in HIV Type 1-Infected Subjects Pre- and Post-Highly Active Antiretroviral Therapy

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ABSTRACT

The T cell receptor excision DNA circle (TREC) level is an independent predictor of HIV-1 disease prognosis. We studied the temporal changes in TREC levels prior to and after highly active antiretroviral therapy (HAART) in a cohort of 131 Greek men with hemophilia who were followed up for up to 20 years since seroconversion (SC). TREC levels were determined in all available cryopreserved samples of peripheral blood mononuclear cells (PBMCs) using a multiplex real-time polymerase chain reaction (PCR) assay. Trends in $\log_{10}$ TREC values were described using random effects models. Prior to HAART initiation TREC levels tended to decrease over time (mean rate of drop 19% per year; 95% CI: 16–22%). Initial TREC values were higher with younger age at SC, but the subsequent rate of drop did not differ significantly by age at SC. There was a monotonic relationship between baseline HIV-RNA levels and TREC slopes with steeper slopes at higher levels of HIV-RNA. The TREC slopes differed significantly by clinical outcome being steeper in subjects who progressed to AIDS sooner. After HAART initiation, TREC values tended to increase on average by 35% per year (95% CI: −7–94%) but the increase was evident only in subjects with a pre-HAART CD4 count below 80 cells/μl. TREC values, which likely represent a simple indicator of naive T-lymphocyte reserve, may be a clinically useful marker for long-term prognosis of HIV-1 infection and for immune reconstitution after successful HAART.

INTRODUCTION

Untreated HIV-1 infection is characterized by a progressive and relentless fall in CD4 T cell counts. The rate of CD4 cell count drop varies substantially among infected individuals. The loss of CD4 T cells has generally been attributed to HIV-1-mediated destruction of peripheral T cells and hyperactivation of the immune system with failure to maintain thymic homeostatis.[1,2] Many studies have implicated the thymus—the central site at which T-lymphocytes mature—in the pathogenesis of HIV-1 infection.[3–8] Older age at HIV-1 seroconversion is associated with faster disease progression.[9] The relation between the age at HIV-1 infection and the subsequent gradient of the CD4 cell count drop has been delineated[10,11] and the findings have been interpreted as consistent with reduced thymic output observed with age.[12,13]

The concentration of T cell-receptor excision DNA circles (TREC) in the peripheral T cell pool has been used as a marker for recent thymic emigrants. While there is a general agreement that TREC levels decrease in HIV disease and increase after HAART,[7,8] whether TREC levels represent a direct indicator of thymic output or they are predominantly affected by other mechanisms such as cell division, cell death, and longevity of naive cells is under debate.[7,8,14–16] In another study[17] we reported that the concentration of TRECs in peripheral blood mononuclear cells (PBMC) was predictive of HIV-1 disease progression.

A few studies have been carried out in a limited number of HIV-1-infected individuals post-HAART.[7,8,14,17–19] A rapid and sustained increase in thymic output was observed in most subjects after HAART.[7] However, Zhang et al.[8] noted that TREC levels were significantly increased with HAART only in patients with low pretreatment values.

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Despite the growing number of studies involving TREC concentrations in HIV-1 infection, no studies have focused on the entire natural history of HIV-1 infection. In the present study, longitudinal TREC measurements from 131 HIV-1-infected hemophiliac men with known seroconversion dates were obtained from stored PBMC specimens to assess the longitudinal trends of TRECs before and after the initiation of HAART.

MATERIALS AND METHODS

Study participants

All clinical samples were obtained from the 158 HIV-1-infected Greek hemophilic men enrolled in the Multicenter Hemophilia Cohort Study. All patients have known seroconver-
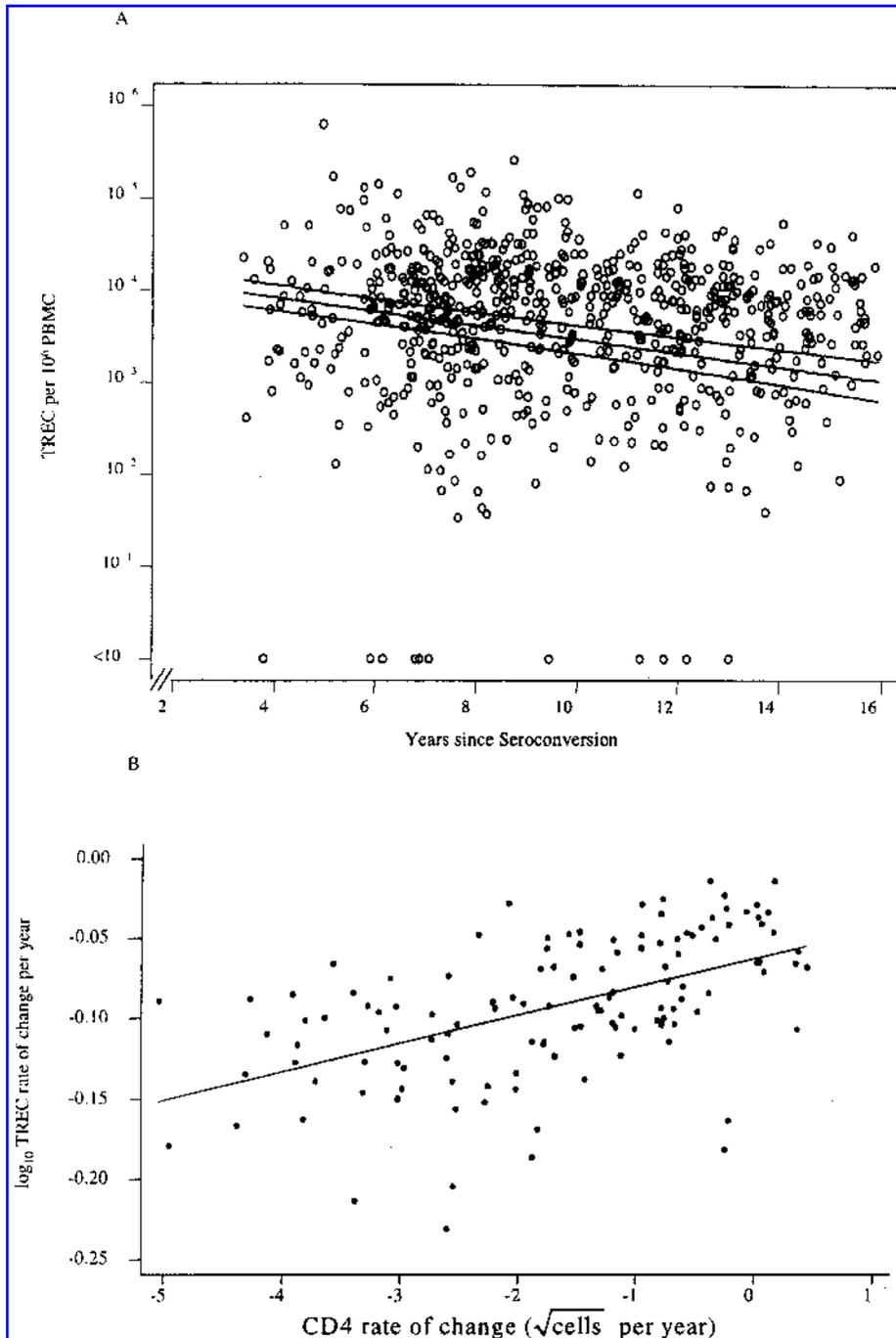

FIG. 1. Average trajectory of TREC levels ($\log_{10}$ scale) in the pre-HAART and pre-AIDS HIV-1 infection period (**A**) and correlation of rate of change in TREC levels ($\log_{10}$ scale) with rate of change in CD4 cell counts (square root scale) (**B**).

sion (SC) dates, and they have been prospectively followed up for up to 20 years since seroconversion. Details on the study population have been presented elsewhere.[10,17]

Clinical and laboratory data have been collected roughly every 6 months. For 131 study participants, at least one cryopreserved sample of PBMCs was available during the follow-up. TREC concentration was measured in all available samples. Plasma HN-RNA during chronic infection was measured from frozen ($-70°C$) serum specimens collected closest to the HIV-1 seroconversion date. The median time between HIV-1 seroconversion and HIV-RNA determination was around 7 years (range: 4–11 years).

Procedures

Plasma HIV-RNA was measured with the ultrasensitive HIV-1 Amplicor Monitor assay (Roch Diagnostics, Alameda, CA), which has a detection limit of 40 HIV-1 RNA copies/ml. CD4 T cell counts were measured by flow cytometry with standard procedures. TREC measurements in PBMCs were obtained by using a multiplex-beacon-based real-time polymerase chain reaction (PCR) assay,[8,17] which quantifies simultaneously the TREC value and the cell equivalent in the input DNA in copies/10^6 PBMCs.

Statistical analysis

TREC data were analyzed separately in the pre- and post-HAART period. Trends in TREC values were described by fitting random-effects (RE) models. These models provide estimates of average marker trends over time while accounting for correlation of repeated measurements within each individual. To linearize changes over time the $\log_{10}$ were used for TREC values. Since AIDS development and the initiation of HAART are expected to affect secular trends in TREC natural history, all TREC data collected after initial AIDS diagnosis or HAART initiation were censored. Clinical AIDS cases were defined in accordance with category C of the 1993 revised classification system of the U.S. Centers for Diseases Control and Prevention[20] while HAART was defined as any protease inhibitor or nonnucleoside analogue reverse transcriptase-based regimen. For the post-HAART analysis, TREC data were censored at the date a successful second line HAART regimen was initiated.

For the natural history analysis, incomplete TREC data due to death or disease progression are likely to be informative and, if so, estimates from the RE models may be biased.[21,22] Specifically, subjects who progress to AIDS earlier tend to have fewer

TREC measurements and thus less stable subject-specific estimates of decline than those with a prolonged AIDS-free period. If subjects who develop AIDS earlier also tend to have steeper rates of TREC decline, the mean rate of TREC loss, estimated by the RE models, will also be biased downward. To adjust for selective (informative) drop-outs the Joint Multivariate RE (JMRE) model was applied.[23] The JMRE method combines a linear RE model for the underlying pattern of TREC trends with a log-normal survival model for the AIDS or death-related drop-outs. The JMRE model, by implying that log survival or AIDS-free times are a function of the subject-specific TREC trends, provides estimates of the correlation between individual TREC trajectories and clinical outcomes. Similar RE models were applied to model longitudinal CD4 data.

RESULTS

Trends in TREC levels prior to HAART initiation

The majority (57.3 %) of the 131 subjects had severe type A hemophilia. Their mean (SD) age at SC was 23.8 (13.9) years ranging from 0.5 to 61.6 years. The median time between HIV-1 SC and first TREC determination (study entry) was 6.6 years (range: 3.4–12.9). Three participants had developed AIDS by the time of the first TREC measurement and they were excluded from the current analysis. For the 128 participants without clinical AIIDS at study entry the median value of TREC was 6,910 copies/10^6 PBMCs [interquartile range (IQR): 2,427–13,251] while the median value of HIV-RNA at study entry was 17,450 (IQR: 4,555–59,515) copies/ml.

At study entry, TREC values were positively correlated with concurrent CD4 cell counts (Spearman $r = 0.36$, $p < 0.0001$) and negatively correlated with age at study entry ($r = -0.20$, $p = 0.029$).

In the pre-HAART period, the median time from SC to the last TREC measurement was 12 years (range: 3.8–18.0). On average, six TREC measurements per subject were taken with a median interval of 1 year between any two successive measurements. By the end of the follow-up 54 subjects had developed AIDS and 16 had died without a diagnosis of AIDS.

The overall mean slope of TREC values (change in $\log_{10}$ copies/10^6 PBMC) was -0.092 per year (95% CI: -0.073, -0.110) corresponding to a 19% (95% CI: 16%, 22%) rate of drop in the original scale. In Figure 1A the scatterplot of TREC values over time after SC is presented. The rate of TREC de-

TABLE 1. RATE OF CHANGE IN LOG_{10} TREC COPIES PER 10^6 PBMCS PER YEAR PER HIV-RNA QUARTILE FROM SEROCONVERSION TO THE DEVELOPMENT OF AIDS OR HAART INITIATION

HIV-RNA quartile (copies/ml)		Mean	95% CI	p value
First	(136–4,555)	-0.073	(-0.097, -0.048)	<0.001[a]
Second	(4,556–17,450)	-0.080	(-0.106, -0.054)	<0.001[a]
Third	(17,451–59,515)	-0.133	(-0.163, -0.104)	0.573[a]
Fourth	(59,516–542,120)	-0.144	(-0.175, -0.113)	<0.001[b]

[a]Comparing subjects in this group with subjects with HIV-RNA in the fourth quartile.
[b]Comparing with zero change.

cline was correlated significantly with each patient's rate of CD4 cell count decrease (Spearman $r = 0.57$, $p < 0.0001$, Fig. 1B).

After adjusting for differences in TREC values at study entry by age, age did not significantly affect the subsequent rate of drop in the $\log_{10}$ scale, indicating that the proportional changes over time were similar among the different age groups. On the contrary, there was a monotonic relation between HIV-RNA levels at study entry and subsequent TREC slopes, with steeper slopes at higher levels of HIV-RNA. TREC values declined by 0.033 $\log_{10}$ copies per 10^6 PBMCs (95% CI: 0.017, 0.049) per 1 $\log_{10}$ copies/ml increase in HIV-RNA levels (Table 1). Figure 2A shows the estimated median TREC trajectories by HIV-RNA quartile at study entry. The association between TREC slopes and HIV-RNA levels remained even after controlling for patient's rate of CD4 decline. In addition, subject-specific TREC slopes were strongly and positively correlated with survival or AIDS-free times ($r = 0.538$, $p = 0.001$) indi-
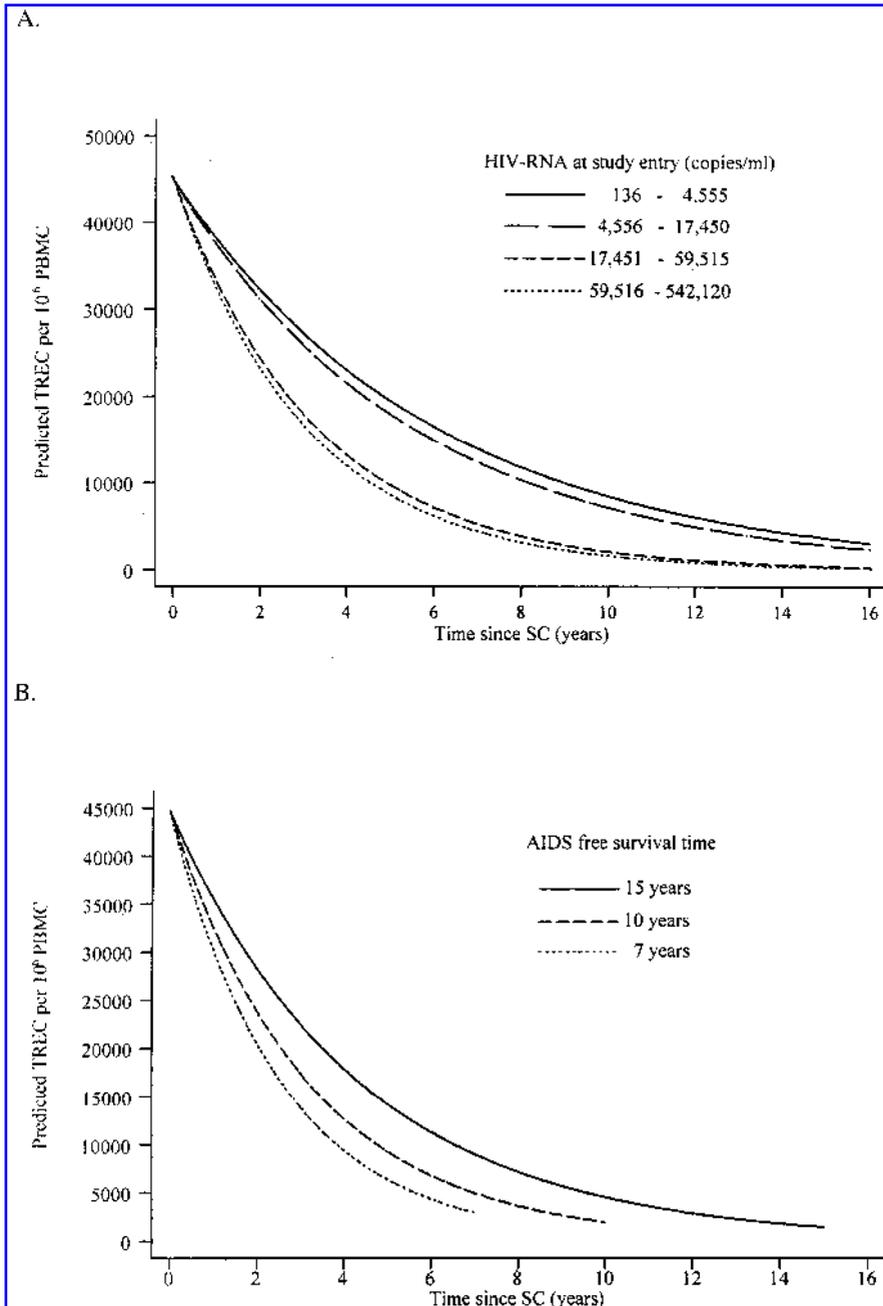

FIG. 2. Average trajectories of TREC levels by HIV-RNA level during chronic HIV-1 infection (**A**) and by time to AIDS or death (7, 10, or 15 years since seroconversion) for subjects with age at seroconversion 15–24 years and HIV-RNA levels during chronic HIV-1 infection 4.29 $\log_{10}$ copies/ml (**B**).

cating that steeper TREC declines are related to poorer clinical prognosis. This relation was found after adjustment of both TREC values and survival times for age at SC and HIV-RNA levels at study entry. Figure 2B presents the estimated median TREC trajectories for subjects who developed AIDS or died at 7, 10, or 15 years after SC.

Of the 57 subjects who progressed to clinical AIDS prior to HAART initiation, 45 had at least one TREC measurement within 1 year prior to AIDS onset (baseline value). For those, the mean (SD) baseline $\log_{10}$ copies per 10^6 PBMCs TREC level was 2.90 (0.93). In subjects not treated with HAART, TREC slopes after the development of AIDS were significantly steeper than before AIDS development. After AIDS onset, the average annual rate of TREC drop (95% CI) was 0.441 (0.267, 0.616) in the $\log_{10}$ scale corresponding to a 64% (46%, 76%) yearly loss in the original scale. However, the rate of TREC change after the development of AIDS was not significantly related to age at SC, age at AIDS development, years from SC

to AIDS development, or AIDS-defining condition (data not presented).

Trends in TREC values after the initiation of HAART

Fifty-one of the 57 patients who initiated HAART during the follow-up period and had at least one TREC value 1 year prior to HAART initiation (baseline value) and at least one additional measurement during the follow-up were included in this part of the analysis. Subjects started HAART at a median time of 14.7 years after SC (range: 11.8–17.3 years). For the majority of the study population ($n = 44$) HAART regimen was based on PIs. The median follow-up time after HAART initiation was 1.5 years ranging from 1 month to 3.6 years. On average four measurements (including the baseline one) of TREC values per subject were taken with the median time being 0.5 years between any two adjacent measurements.
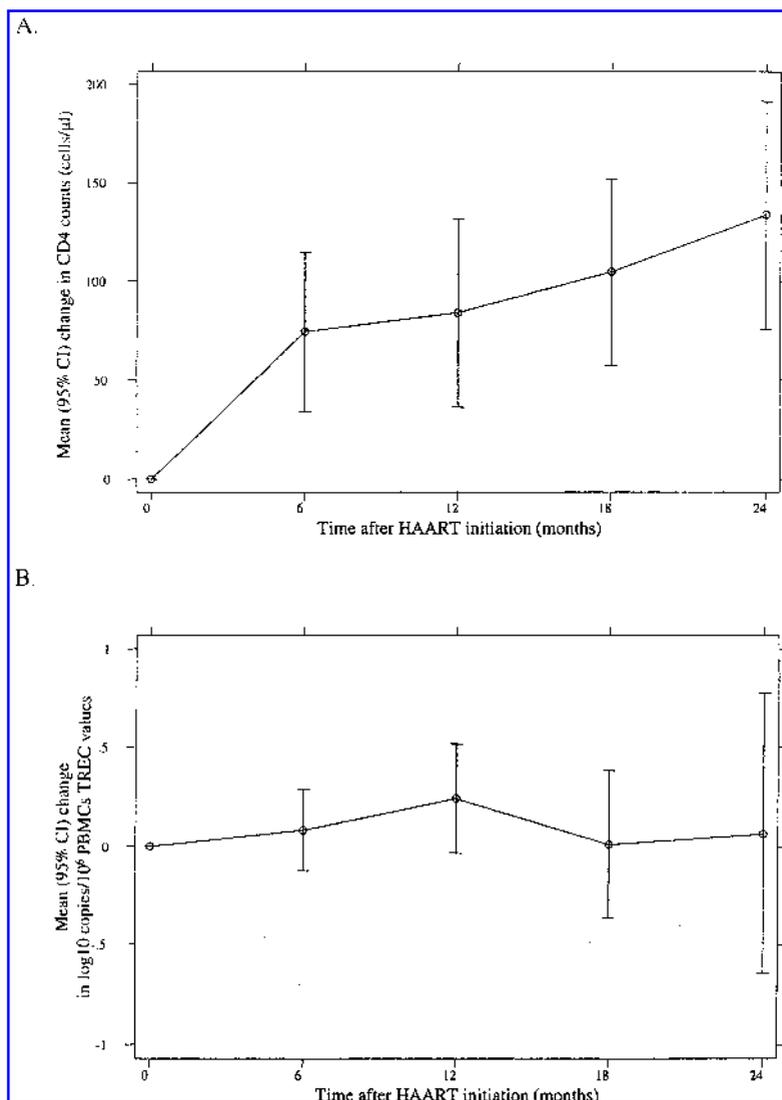

FIG. 3. Cross-sectional mean (95% CI) changes in $\log_{10}$ TREC values (**A**) and CD4 cell counts (**B**) during the first 2 years after HAART initiation.

The mean (SD) value of pre-HAART $\log_{10}$ copies/10^6 PBMCs TREC and $\log_{10}$ copies/ml HIV-RNA was 3.37 (0.80) and 4.11 (0.90), respectively, while the median (IQR) pre-HAART CD4 cell count was 149 (80–289) cells/μl. Pre-HAART TREC values were significantly correlated with CD4 cell counts (Spearman $r = 0.54$; $p < 0.001$). Subjects having already developed AIDS tended to have lower TREC values but after adjustment for concurrent CD4 cell count this difference was insignificant. Figure 3 shows the cross-sectional mean (95% CI) changes in $\log_{10}$ TREC values (Fig. 3A) and CD4 cell counts (Fig. 3B) during the first 2 years after HAART initiation.

On average, TREC values tended to increase after HAART initiation with the mean (95% CI) rate of change per year in $\log_{10}$ copies/10^6 PBMCs TREC values being 0.129 (-0.029, 0.287) corresponding to a 35% (-7%, 94%) rate of increase in the original scale. The mean increase in CD4 cell count was 32 cells/μl per year (95% CI: 14, 50). In the majority of subjects (31 of 51; 60.1%) an increase in both CD4 cell count and TREC levels was observed after HAART initiation. However, 18 (35.3%) subjects had discordant TREC and CD4 slopes, with positive TREC slopes and negative CD4 slopes in 15 of the 18 subjects. Factors such as age at SC or HAART initiation, time from SC to HAART initiation, AIDS status and baseline CD4, and HIV-RNA levels were examined for potential associations with TREC rate of change in univariate and multivariate models. The only baseline factor significantly related with TREC slope was CD4 cell count. More specifically, TREC values increased significantly only in subjects with CD4 cell counts less than 80 cells/μl (first quartile of the corresponding distribution) while there was a monotonic decrease in TREC slope for subjects with a higher baseline CD4 cell count (Table 2). TREC slope was also positively correlated with the subject-specific rate of CD4 cell count change even after adjusting for baseline CD4 cell count ($p = 0.030$). However, virological response (HIV-RNA load <50 copies/ml) was inversely related to TREC increase after adjusting for baseline CD4 and rate of CD4 change but this relation was not significant ($p = 0.349$).

DISCUSSION

In this study we observed a positive correlation between TREC levels in PBMC and CD4 cell count and an inverse relation with age (both at study entry and at HAART initiation) as reported elsewhere.[7,8,24–26] We previously reported that HIV-1-infected adults have on average lower TREC levels than age-matched HIV-1-uninfected adults but a significant overlap in the distribution of values was observed between the two groups.[8,17] Instead, a more prominent impact of HIV-1 infection on TRECs is reported in infected children, approximately half of whom have values below the tenth percentile for healthy children.[8]

Using longitudinal TREC measurements during the natural history of HIV-1 infection, we found that TREC concentrations tended to decrease over time during chronic HIV-1 infection. The estimated overall mean rate of TREC drop per year (-0.092 in $\log_{10}$ scale) was more than 2.5 times steeper than that reported in HIV-uninfected adults.[8,17] This is consistent with previous findings of reduced TREC levels in HIV-1 infection.[7,8,17] In addition, in multivariate analysis we found that the higher the HIV-RNA level during chronic infection the greater the rate of subsequent decline in TREC levels. This additional effect of HIV-RNA may reflect a dilution of TREC-containing cells due to persistent activation of the immune system[15,18] but a direct effect of HIV-1 infection on thymus impairment cannot be excluded.[14,16,25–28] Of note, TREC decreases were strongly associated with decreases in CD4 cell count. Moreover, the rate of TREC decline was associated with the time to AIDS development or death. It also is noteworthy that TREC values in long-term nonprogressors remained almost stable for many years after HIV-1 SC.[17] The association between the rate of TREC decline and time to AIDS remained even after adjusting for baseline HIV-RNA levels and age at study entry. This finding is consistent with our previous analysis[17] that concentrations of TREC in early PBMC samples complement HIV-RNA load in predicting the rate of HIV-1 disease progression.

A further acceleration of TREC decline was observed after AIDS development. This is consistent with data suggesting highly increased immune activation around the time of AIDS development[29] as well as the appearance of HIV-1 strains with CXCR4 tropism that may further reduce thymic output by infection of naive T cells.[30–32]

Douek et al.[7] found that there was a rapid and sustained increase in TREC values in adults treated with HAART. This finding was interpreted as suggesting that HAART improved thymic function. Instead, Zhang et al.[8] and Franco et al.[33] found that TRECs increased after HAART primarily in patients with an existing TREC impairment, while in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial[25] it was shown that change in TREC level after ART was inversely related to baseline CD4 cells. This is consistent with our results that TRECs were substantially increased after HAART mainly in subjects with very low CD4 cell counts (<80 cells/mm^3) at HAART initiation. This finding may also reflect partial restora-

TABLE 2. RATES OF CHANGE IN TREC VALUES ($\log_{10}$ COPIES/10^6 PBMCS) AFTER HAART INITIATION BY CD4 CELL COUNT LEVEL

CD4 count (cells/μl)	N	Average rate of change per year in $\log_{10}$ TREC	95% CI
0–79	13	0.428	(0.221, 0.635)
80–149	13	0.176	(-0.088, 0.441)
150–288	13	-0.050	(-0.396, 0.297)
≥289	12	-0.377	(-0.757, 0.003)

tion of thymic output as well as an abrupt decrease of immune activation of T cells after initiation of HAART.[14] In a recently published study, Lewin et al.,[16] using a mathematical model that accounts for proliferation, death, and redistribution of T cells, found that an increase in thymic output is needed to explain the increase in TREC during treatment of chronic HIV infection. The implications of discordant changes of CD4 and TREC in 35% of our patients after initiation of HAART are not clear. The initial rise of memory T cells due to redistribution of T cells immediately after HAART may dilute TRECs.[2] Alternatively, HAART may not be sufficient to reduce immune activation in some patients resulting in no TREC increase.

Results regarding the effect of age at initiation of therapy on TREC changes are contradictory.[25,28] In our longitudinal study it was found that apart from the difference in baseline TREC values by age group, age did not significantly influence subsequent TREC changes both during the natural history and after HAART initiation. This finding suggests that starting HAART at any age can result in increased thymus output. This is consistent with recent data showing immunological response in subjects treated with HAART irrespective of age.[25,33–35]

In conclusion, this analysis, taken together with previous studies, suggests that TREC levels in HIV-1-infected individuals may be a surrogate marker of thymic output, although in advanced HIV-1 infection with increasing immune activation due to continuously elevated HIV-1 replication, TREC levels may mostly reflect hyperactivation of the immune system rather than thymic output. In either case, TREC remains a simple marker of naive T-lymphocyte reserve in the peripheral blood and may prove to be clinically useful for estimating the prognosis of HIV-1 infection and the success of immune reconstitution after HAART.

ACKNOWLEDGMENTS

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