Evidence for Increased T Cell Turnover and Decreased Thymic Output in HIV Infection


*J Immunol* 2001; 167:6663-6668; doi: 10.4049/jimmunol.167.11.6663

http://www.jimmunol.org/content/167/11/6663

**References** This article cites 50 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/167/11/6663.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Evidence for Increased T Cell Turnover and Decreased Thymic Output in HIV Infection


The effects of HIV infection upon the thymus and peripheral T cell turnover have been implicated in the pathogenesis of AIDS. In this study, we investigated whether decreased thymic output, increased T cell proliferation, or both can occur in HIV infection. We measured peripheral blood levels of TCR rearrangement excision circles (TREC) and parameters of cell proliferation, including Ki67 expression and ex vivo bromodeoxyuridine incorporation in 22 individuals with early untreated HIV disease and in 15 HIV-infected individuals undergoing temporary interruption of therapy. We found an inverse association between increased T cell proliferation with rapid viral recrudescence and a decrease in TREC levels. However, during early HIV infection, we found that CD45RO+CD27high (naive) CD4+ T cell proliferation did not increase, despite a loss of TREC within naive CD4+ T cells. A possible explanation for this is that decreased thymic output occurs in HIV-infected humans. This suggests that the loss of TREC during HIV infection can arise from a combination of increased T cell proliferation and decreased thymic output, and that both mechanisms can contribute to the perturbations in T cell homeostasis that underlie the pathogenesis of AIDS. The Journal of Immunology, 2001, 167: 6663–6668.

The measurement of TCR rearrangement excision circles (TREC) has been used to assess thymic output in individuals with and without HIV infection (23, 36, 34–37), and after hematopoietic stem cell transplantation (29, 38, 39). In the majority of individuals with untreated HIV-infection, TREC levels were below normal, but increased after viral suppression with HAART (23, 26, 37). This was taken to indicate that the thymus, in both adults and children, is suppressed by HIV infection, but contributes to T cell

---

Copyright © 2001 by The American Association of Immunologists

0022-1767/01/$02.00

Received for publication June 5, 2001. Accepted for publication September 24, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by the National Institutes of Health (Grants AI35522 and AI43638 to R.A.K. and Grant AI43638), by the University of California (San Diego) Cancer Research and Development Center, Frederick, MD 21702; and by the Leukemia and Lymphoma Society of America (Translational Research Grant 6540-00), and by amFAR (Grant 02680-28-RGV to D.C.D.). This project has also been funded in part with Federal funds from the National Cancer Institute under Contract NO1-CO-56000.

3 Abbreviations used in this paper: HAART, highly active antiretroviral therapy; BrdU, bromodeoxyuridine; TREC, TCR rearrangement excision circles.
reconstitution during HAART. In this study, we sought to determine whether increased T cell turnover, decreased thymic output, or both occur in HIV infection. We measured peripheral blood TREC levels and parameters of CD4$^+$ and CD8$^+$ T cell proliferation, including Ki67 expression and ex vivo BrdU incorporation, in 22 individuals with early untreated HIV disease (3–12 mo after infection), and in 15 successfully treated HIV-infected individuals who underwent temporary interruption of therapy.

Materials and Methods

**Human subjects**

Twenty-two patients with early HIV infection (3–12 mo after seroconversion) were seen at the University of Texas Southwestern Medical Center and had not been on antiretroviral drugs at the time of blood draw. CD4$^+$ T cell counts were 220-1080 cells/μl (mean 602) and viral loads were <400 to >7.5 × 10^8 RNA copies/ml. Fifteen patients were asymptomatic HIV-infected adults with baseline CD4$^+$ T cell counts of >350 cells/μl who had been on continuous HAART for a minimum period of 1 year, with viral loads consistently below the limits of detection for at least that period of time (40). On day 0, patients discontinued all antiretroviral drugs, and resumed drugs when any of the following three conditions was met: the CD4$^+$ T cell count declined at least 25% from the mean of three baseline determinations, their viral load increased to 5000 RNA copies/ml, or the patient resumed drug treatment independently. Viral loads were measured by Amplicor assay (Roche, Basel, Switzerland). Studies were approved by the Institutions’ review boards, and patients gave informed consent.

**Measurement of TREC in MACS-sorted cells**

Quantification of TREC in sorted CD4$^+$ and CD8$^+$ T cells was performed by quantitative PCR with an ABI7700 system (PerkinElmer/Cetus, Norwalk, CT) as previously described (29). PBMC were separated into CD4$^+$ and CD8$^+$ cells using MACS microbeads (Miltenyi Biotec, Auburn, CA). Cells were lysed in proteinase K (Boehringer Mannheim, Indianapolis, IN) and PCR was performed on 5 μl of cell lysate (50,000 cells). A standard curve was plotted, and TREC values for samples were calculated by the ABI7700 software. Samples were analyzed in duplicate. TREC levels are expressed as TREC per microgram of DNA (1 μg of genomic DNA is equivalent to 150,000 cells). Cell lysates have been checked for consistency of DNA content using β-actin and CCR5 control PCR; interassay variability was found to be less than 13% of mean for the same sample in 20 different assays (data not shown).

**Ex vivo BrdU uptake analysis**

Blood samples were incubated with 100 μM BrdU for 4 h at 37°C. Cell surface staining was performed using Abs to CD3, CD45RO, CD4, and/or CD8 (BD Biosciences, San Jose, CA). Cells were treated with OptiLyse (ImmunoTech, Westbrook, ME) for 10 min at room temperature, then with 1% paraformaldehyde and 1% Tween 20 in PBS for 15 min at 37°C. Cells were lysed in proteinase K (Boehringer Mannheim) for 30 min and were then stained with anti-BrdU-FITC (BD Biosciences). Events (50,000–100,000) were collected flow cytometrically, resulting in a sensitivity of 0.01% BrdU$^+$ events, and were analyzed in parallel with unlabeled cells from the same individual and this value was subtracted from the value obtained for BrdU-labeled cells. Data are expressed as the fold change in the percentage of BrdU$^+$ cells to avoid large baseline differences in absolute values between individuals. However, similar statistical significance was obtained when the fold change in absolute BrdU$^+$ cells was used (data not shown).

**Naive T cell Ki67 analysis and FACS sorting**

Analysis was performed by surface staining cells for either CD4/CD45RO/CD27 or CD8/CD45RO/CD27 (BD Biosciences), followed by fixation/permeabilization and intracellular staining for Ki67 (BD Biosciences). Cells were analyzed by four-color flow cytometry using a FACS Calibur. Ki67 expression was measured in both CD45RO$^+$ CD27$^{high}$ (naive) and CD45RO$^+$ (memory/effector) CD4$^+$ and CD8$^+$ T cells using a FACS Canto (BD Biosciences).

**Statistical analyses**

Two-tailed Mann-Whitney U test and Spearman’s rank correlation coefficients were performed using SAS and Prism software (SAS Institute, Cary, NC). Analysis of covariance was used to assess differences in CD4$^+$ and CD8$^+$ T cell TREC between HIV-infected individuals and the healthy controls, adjusting for age. A p value of <0.05 was considered significant.

**Results**

**TREC levels measure thymic output**

TREC frequency in total and in naive T cells has been shown to decrease with age, after thymectomy, and in HIV infection (23, 26, 34–36, 41). However, a recent mathematical model has suggested that this decrease in TREC solely reflects a theoretical increase in the naive T cell division rate, and not decreased thymic output (42). Therefore, we sought to test this experimentally by measuring changes in T cell division with age using Ki67 expression as a surrogate marker of cell proliferation. Fig. 1 shows that Ki67 expression does not increase in either CD4$^+$ or CD8$^+$CD45RO$^+$ CD27$^{high}$ (naive) T cells in healthy individuals aged between 23 and 88 years of age (during which period the most rapid drop in naive T cell TREC is seen; Refs. 23 and 26). Furthermore, it has recently been shown that although TREC decrease after thymectomy, there is no increase in CD27$^{high}$ (naive) or CD45RO$^+$ memory T cell Ki67 expression (41). The fact that Ki67 may be raised in nonproliferating or activated cells (3, 43) does not affect this analysis because the aim in this study was to exclude any increases in proliferation. Thus, a decrease in TREC levels can indeed reflect a decrease in thymic output.

**TREC levels and BrdU uptake after interruption of therapy**

It has recently been shown that after total thymectomy, TREC levels begin to fall after only 3 mo (41). Therefore, in HIV infection, any decrease in TREC related to decreased thymic output would not be expected to be observed until at least 3 mo after seroconversion. Consequently, decreases in TREC before 3 mo of HIV infection would reflect primarily increased T cell proliferation.

To examine this, we initially studied T cell TREC levels in 15 HIV-infected individuals who had been successfully treated with HAART, had undetectable viral loads, and then underwent interruption of therapy (40, 44). As previously reported, recrudescence of viral replication occurred in all the patients within 28 days of interruption of therapy. This allowed us to longitudinally assess changes in TREC and proliferation during a rapid rise in HIV levels—a situation reminiscent of acute HIV infection. As viral load rose, both CD4$^+$ and CD8$^+$ T cell TREC levels decreased concomitantly. We then performed ex vivo BrdU incorporation to determine whether this fall in TREC was, in part, secondary to increased T cell proliferation. The correlation between the S-phase BrdU fraction and viral load has been previously described (44). In
the interval between interruption and resumption of therapy, there was a significant negative correlation between the change in the percentage of BrdU$^+$CD4$^+$ T cells and the change in CD4$^+$ T cell TREC levels ($r = -0.6, p = 0.02$, and 95% confidence interval $= -0.86$ to $-0.09$; Fig. 2). Thus, as CD4$^+$ T cell proliferation increased with a concomitant increase in viral load (40), TREC levels decreased. The change in the percentage of BrdU$^+$CD8$^+$ T cells also varied inversely with the change in CD8$^+$ T cell TREC levels. However, this relationship was not statistically significant for this sample size ($r = -0.3, p = 0.3$, and 95% confidence interval $= -0.72$–0.31), and a larger study sample would be required to establish a statistically significant relationship. It is a possibility that preferential redistribution of TREC-containing cells out of the peripheral circulation could cause the decrease in TREC. Of course, these data do not rule out a concomitant decrease in thymic output; it is simply not possible to differentiate the effects of thymic output and T cell proliferation. However, bearing in mind the delayed effects of thymectomy on TREC levels (41), the rapid fall in TREC during an acute rise in HIV load more likely reflects increased T cell proliferation than decreased thymic output.

**TREC levels and Ki67 expression in early HIV infection**

Inhibition of thymic function should become detectable by 3 mo after HIV infection. To differentiate experimentally between thymic inhibition and increased peripheral T cell proliferation, it is necessary to measure an independent marker of T cell proliferation in naive and memory T cells, and to measure or calculate the TREC content of the naive T cell pool. If naive T cell proliferation is constant, then changes in TREC levels reflect changes in the supply of naive T cells. Therefore, we measured TREC levels and Ki67 expression in naive and memory CD4$^+$ and CD8$^+$ T cell subsets in a separate group of 22 patients with early (3–12 mo after seroconversion) untreated HIV infection. Ki67 expression was used, rather than BrdU incorporation, because these were cryopreserved samples. It should be stressed, however, that the number of Ki67$^+$ cells and the S-phase fraction (BrdU$^+$ cells after a 4-h in vitro pulse) are not equivalent measures of T cell activation, and many more cells express Ki67 than are actually in S-phase at any particular instant in time. The longevity of Ki67 expression after mitosis remains unclear.

![FIGURE 2](image)

**FIGURE 2.** Correlation between TREC levels and BrdU incorporation in treated HIV-infected individuals after therapy interruption. Shown are the relationships between the fold change in the percentage of BrdU$^+$CD4$^+$ and BrdU$^+$CD8$^+$ T cells, and the fold change in CD4$^+$ and CD8$^+$ T cell TREC levels in the interval between interruption of therapy and its resumption with high viral load. Best-fit exponential regression curves are shown.

![FIGURE 3](image)

**FIGURE 3.** Early HIV infection and TREC levels. TREC levels in sorted CD4$^+$ and CD8$^+$ T cells from uninfected individuals (○) and untreated individuals with early (3–12 mo after seroconversion) HIV infection (●) are shown. Best-fit exponential regression curves for uninfected individuals are shown.

![FIGURE 4](image)

**FIGURE 4.** T cell Ki67 expression in early HIV infection. The percentage of CD4$^+$ and CD8$^+$ CD45RO$^+$CD27$^+$ (naive) and CD45RO$^+$ (memory) T cells that are Ki67$^+$ in uninfected individuals (−) and individuals with early HIV infection (+) are shown. The top, bottom, and line through the middle of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers extend from the 10th percentile to the 90th percentile.
also significantly increased in HIV-infected individuals (3.6-fold, \( p = 0.0002 \)), the percentage of Ki67+ CD4+ CD45RO− CD27high (naive) T cells did not differ significantly from uninfected individuals (\( p = 0.064 \)), and was in fact slightly lower. Thus, even though Ki67 expression may indicate T cell proliferation and/or activation, these data suggested that the decrease in CD4+ T cell TREC during HIV infection could not be the result of increased turnover of naive CD4+ T cells.

**Naive CD4+ T cell activation in early HIV infection**

Although we found no increase in naive CD4+ T cell Ki67 expression in early HIV infection, other studies have shown it to be increased (31). The phenotypic definition of naive T cells as CD45RO− CD27high by flow cytometry reveals that HIV-infected subjects contain more T cells with a phenotype between that of true naive and effector/memory cells than uninfected individuals (Fig. 5). These have been termed “transitional” cells (45). Therefore, we reanalyzed the data shown in Fig. 4, incorporating a small proportion of transitional CD4+ T cells into the naive T cell gate, and then measuring Ki67 expression. We found that the inclusion of only 5% more transitional cells apparently increased naive CD4+ T cell Ki67 expression 4.7-fold (range 4−16) in the HIV-infected subjects, but only 1.7-fold (range 1.3−2.2) in the uninfected subjects. This difference was statistically significant (\( p = 0.01 \)), and leads to the misleading conclusion that naive CD4+ T cell Ki67 expression is increased in HIV-infected individuals. An example of the effect of inclusion of transitional T cells on naive CD4+ T cell Ki67 expression is shown in Fig. 5. These T cells may have recently been naive cells that are transitioning to activated cells and that will proliferate. Thus, accurate measurement of activation and proliferation in naive CD4+ T cells requires rigorous phenotypic definition of this population by flow cytometry.

**Naive T cell TREC levels in early HIV infection**

Therefore, to determine whether thymic output was decreased in early HIV infection in the context of unchanged naive CD4+ T cell proliferation (and also to exclude memory T cell expansions as a cause of decreased total T cell TREC), we calculated naive T cell TREC from the total measured TREC and the percentage of naive T cells determined by flow cytometry. Our calculation assumed that the contribution of TREC from memory T cells was negligible. This is a valid assumption, as we have measured TREC in highly FACS-purified CD4+ T cell populations from 12 individuals and have found that CD45RO− (memory) T cells have, on average, only 2% of the TREC content of CD45RO+− (naive) T cells in the same individual. Furthermore, as an example of the concordance between calculated and actual measured naive T cell TREC, we FACS sorted CD45RO+− CD27high (naive) T cells in one individual, measured TREC directly, and compared this result with TREC calculated from unsorted T cells and the naive T cell percentage, as shown in Tables I and II.

We found that both CD4+ and CD8+ naive T cell TREC in early HIV infection were significantly lower than in uninfected age-matched controls (\( p = 0.006 \) and 0.001, respectively; Fig. 6a). Because the percentage of Ki67+ CD8+ CD45RO− CD27high (naive) T cells was increased in HIV-infected individuals, the reduced naive CD8+ TREC levels could have been solely or partly due to increased naive CD8+ T cell proliferation. However, the percentage of Ki67+ CD4+ CD45RO− CD27high (naive) T cells was not increased, and therefore, the decrease in TREC within CD45RO− CD27high (naive) CD4+ T cells could not have been due to increased proliferation of cells in this compartment. This suggests that the decreased naive CD4+ T cell TREC levels caused by HIV infection should be reversible when virus replication is suppressed. To confirm this, we longitudinally followed, after initiation of HAART, the CD45RO+− CD27high (naive) CD4+ T cell TREC levels of five patients who had low TREC before therapy. After initiation of HAART, which reduced viral loads to undetectable levels, CD45RO+− CD27high (naive) CD4+ T cell TREC rose to normal levels, suggesting that changes in thymic output were responsible for the changes in TREC within the naive CD4+ T cells before and after HAART (Fig. 6b).

**Discussion**

There is a considerable body of evidence that HIV can infect the thymus, both in vitro and in vivo, and compromise its function (10−14, 16, 46, 47). Recent studies of CD4+ T cell depletion and reconstitution in HIV infection have tended to appreciate the multifactorial nature of immune homeostasis—the composition of naive and memory/effector pools in blood and lymphoid tissue, the role of the thymus, and the effect of clinical stage of the disease.
in therapy, the rapid fall we observed in TREC levels clearly re-
experiencing a rebound of viral replication after interruption of
increased T cell turnover, occur in HIV infection. In individuals
unlikely that the fall in TREC re-

1, 408; 2, 521; 3, 850; 4, 761; and 5, 416.

The measurement of peripheral blood TREC provides insight
into both thymic function and T cell proliferation. If naive T cell
TREC levels decrease in the absence of an increase in prolifera-
tion, it may be concluded that there is a decrease in the supply of
new TREC+ cells, most likely from the thymus. In this study, we
aimed to determine whether decreased thymic output, as well as
increased T cell turnover, occur in HIV infection. In individuals
experiencing a rebound of viral replication after interruption of
therapy, the rapid fall we observed in TREC levels clearly reflects
the increase in T cell proliferation. Although not impossible, it is
unlikely that the fall in TREC reflects a marked decrease in thymic
output, as it has recently been shown that TREC levels only begin
to decrease 3 mo after total thymectomy in HIV-uninfected
individuals (41).

Therefore, we reasoned that if HIV infection suppressed thymic
output, we would be able to detect this effect at least 3 mo after
infection. Indeed, our results from the analysis of individuals with
early HIV infection 3–12 mo after seroconversion suggest that
decreased thymic output begins to affect T cell homeostasis by this
stage of the disease. TREC levels were decreased in both CD4+
and CD8+ T cell subsets, and were also significantly decreased
when the CD45RO−CD27high (naive) T cell TREC were calcu-
lated. As both CD45RO+ (memory) and CD45RO−CD27high (naive)
CD8+ T cell populations had higher Ki67 expression in HIV-infected
individuals, it was not possible to distinguish between the effects
of increased proliferation and reduced thymic output for CD8+ T cells.
However, the percentage of Ki67+ CD4+CD45RO−CD27high (naive)
T cells did not increase. The finding that TREC within naive CD4+
T cells were significantly lower in infected individuals in the con-
text of unaltered naive CD4+ T cell proliferation suggests that the
input of TREC+ naive CD4+ T cells into the peripheral naive T cell
cell pool from a “source” has decreased. The thymus is the most
likely source for such cells (50).

However, some studies have found that the percentage of
Ki67+ CD4+ naïve T cells increased in HIV-infected individuals
(1, 31). The discrepancy between our data and these studies could
be due a number of reasons; for example, our subjects had a higher
mean CD4 counts, and some Ki67+ cells might be nondividing (3, 43).
However, as we have shown, it is more likely that the incor-
poration of transitional T cells, cells that were naive and have now
become activated, into the phenotypically defined naïve T cell
subset accounts for the apparent increase in CD4+ naïve T cell ac-
tivation and/or proliferation in such studies.

Thus, our data show that by 3 mo after HIV infection, a decrease
in TREC within CD45RO−CD27high (naive) CD4+ T cells is clearly
detectable in the absence of an increase in CD45RO−CD27high (na-
ive) CD4+ T cell proliferation, which suggests a decrease in thy-
mic output. It is possible that the decrease in TREC could be due
to members of the naïve pool entering cell cycle, losing TREC due
to dilution, and then reverting to a naïve phenotype. However,
there is no evidence in humans for activated or memory CD4+ T
cells reverting to a CD45RO−CD27high phenotype. The effect of
HIV on thymic function was further confirmed with the observa-
tion that in those individuals with low TREC, CD45RO−CD27high
(naive) CD4+ T cell TREC increased with suppression of virus on
HAART. This suggests that the thymus can recover from its sup-
pression during HIV infection. It is important to note that an in-
crease in naïve T cell TREC can only occur in the context of active
thymic output. Therefore, even if decreases in CD4+ T cell TREC
occurred due to proliferation and the thymus remained unaffected
by HIV, the increase in naïve T cell TREC during HAART indi-
cates that the thymus contributes to immune reconstitution. An
appreciation of the relative roles of the thymus and peripheral T
cell pool in immune reconstitution in HIV infection may provide a
framework for the rational design of interventions that accelerate
and improve the nature of T cell reconstitution in HIV-infected
individuals.

Acknowledgments

We thank Dr. J. Sullivan for samples and Drs. L. Picker and Z. Grossman
for advice.

### Table II. TREC levels per 10⁶ cells in CD45RO−CD27high (naive) and CD45RO+ (memory) T cell subsets measured and back-calculated in one individual

<table>
<thead>
<tr>
<th>% CD45RO−CD27high</th>
<th>MACS-Sorted CD4+ T Cell TREC</th>
<th>Calculated Naive CD4+ T Cell TREC</th>
<th>FACS-Sorted Naive CD4+ T Cell TREC</th>
<th>FACS-Sorted Memory CD4+ T Cell TREC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.06%</td>
<td>692</td>
<td>2,032</td>
<td>2,054</td>
<td>6</td>
</tr>
</tbody>
</table>

**FIGURE 6.** CD45RO−CD27high (naive) T cell TREC in early HIV infec-
tion and its treatment. a. CD45RO−CD27high (naive) T cell TREC lev-
els in sorted CD4+ and CD8+ T cells from uninfected individuals (○) and
individuals with early HIV infection (●), calculated from the percentage
of CD45RO−CD27high (naive) T cells present in each population. Best-fit
exponential regression curves for uninfected individuals are shown. b.
Increases during HAART in CD45RO−CD27high (naive) CD4+ T cell TREC
in five patients indicated in a who had low TREC before therapy. CD4+ T
cell counts per microliter at the start of treatment for the five patients were:
1, 408; 2, 521; 3, 850; 4, 761; and 5, 416.
References


