Distinct Mechanisms of T Cell Reconstitution Can Be Identified by Estimating Thymic Volume in Adult HIV-1 Disease

Robert C. Kalayjian,1,2 John Spritzler,2 Minya Pu,3 Alan Landay,4 Richard B. Pollard,6 Vicki Stocker,4 Lena-Al Harthi,4 Barry H. Gross,7 Isaac R. Francis,4 Susan A. Fiscus,8 Pablo Tebas,9 Ronald J. Bosch,3 Victor Valcour,10 and Michael M. Lederman,2 for the Adult AIDS Clinical Trials Group 5015 and 5113 Study Teams

1Division of Infectious Diseases, MetroHealth Medical Center and 2Case Western Reserve University, Cleveland, Ohio; 3Harvard School of Public Health, Boston, Massachusetts; 4Rush Medical College, Chicago, Illinois; 5University of California, Davis, Sacramento; 6Social and Scientific Systems, Inc., Silver Spring, Maryland; 7University of Michigan, Ann Arbor; 8University of North Carolina, Chapel Hill; 9University of Pennsylvania, Philadelphia; 10University of Hawaii, Honolulu

Background. We have attempted to identify factors associated with T cell reconstitution in response to highly active antiretroviral therapy.

Methods. In a prospective, multicenter cohort study, we compared clinical, immune, and viral responses to an initial antiretroviral regimen in older (≥45 years old) versus younger (18–30 years old) human immunodeficiency virus type 1–infected subjects. Multivariable linear-regression models identified independent factors associated with changes in T cell counts.

Results. Older subjects had smaller increases in naive T cells but greater T cell receptor–excision circle DNA content after 48 weeks, despite similar virologic responses and comparable reductions in immune activation. Changes in T cell counts were associated with plasma interleukin (IL)–7 levels in subjects with low thymic scores, whereas first-phase T cell increases (perhaps mediated by redistribution to the circulation of tissue-associated lymphocytes) were associated with reductions in immune activation in subjects with high thymic scores. Reductions in immune activation were associated with reductions in spontaneous lymphocyte apoptosis.

Conclusions. Distinct processes may underlie T cell restoration, according to estimated thymic volumes. IL-7–mediated peripheral expansion may drive T cell restoration in persons with low thymic volume, whereas therapy-associated reversal of immune reactivation may drive T cell losses and their restoration in persons with larger thymic volume.

Progressive CD4+ cell depletion, with disproportionate declines in naive CD4+ and CD8+ cells, characterizes untreated HIV-1 disease [1, 2]. The mechanisms that underlie these declines and the processes responsible for T cell reconstitution in association with highly active antiretroviral therapy (HAART) remain incompletely understood. In addition to the direct cytopathic effects of viral replication on T cells, the relative contributions to T cell homeostasis of thymic dysfunction, accelerated cell losses via activation-associated apoptosis, and peripheral cytokine–dependent T cell expansion remain poorly defined in HIV-1 disease [3, 4].

Older age is a strong predictor of accelerated HIV-1 disease progression and mortality, as has been demonstrated in studies that were conducted both before and after the availability of HAART [5–12]. As with HIV-1 disease, naive T cell depletion is a hallmark of age-related immunosenescence [13, 14]. It is therefore not surprising that older HIV-1–infected persons demonstrate impaired naive T cell reconstitution in response to HAART [15, 16]. Although age-associated reductions in thymic function may account for these impairments [17, 18], the importance of thymic dysfunction in HAART-associated T cell restoration re-
mains controversial [3]. Although increases in numbers of T cells have been correlated with markers of thymic function, including radiographically estimated thymic volumes and T cell receptor excision circles (TRECs) [19–25], naive T cell expansion has been demonstrated in the absence of thymic output [26, 27], and defects in T cell production have not been consistently identified in lymphocyte kinetics studies that used direct labeling techniques [28–30].

To better understand the mechanisms underlying age-associated accelerated progression of HIV-1 disease, we compared detailed immune and viral responses to an initial HAART regimen in older versus younger HIV-1–infected adults who enrolled in a prospective, multicenter cohort study. Baseline cross-sectional comparisons of this cohort, which included age-matched, healthy control subjects, have been reported elsewhere [31]. We report here an analysis of immunologic and viral outcomes after 48 weeks of HAART.

The heterogeneity of thymic function that characterized the cohort also represented an opportunity to investigate the importance of thymic contributions to T cell restoration. Because thymic-dependent and -independent pathways of T cell regeneration have been demonstrated in animal studies [32], we hypothesized that the relative contributions of viral replication, immune activation, and interleukin (IL)—7—a lymphotropic cytokine—to T cell reconstitution also may differ according to thymic function. To test this hypothesis, we examined the relationships of these factors to changes in T cell counts according to thymic score, a radiographic estimate of thymic volume, using multivariable linear-regression modeling. Models for first-phase (weeks 0–12), second-phase (weeks 12–48), and overall (weeks 0–48) changes in T cell counts were compared to distinguish factors associated with early T cell redistribution to the circulation of lymph node– and lymphoid organ–associated lymphocytes from factors associated with longer-term changes.

SUBJECTS, MATERIALS, AND METHODS

Study design and subjects. The present study was a multicenter, prospective cohort study of older (∼45 years old) and younger (18–30 years old) HIV-1–infected adults who had not previously received HAART. These ages were selected because differences in outcome in adults have been demonstrated to emerge by the third decade of life [5, 6, 9, 11, 12]. Participants in the cohort had CD4+ cell counts <600 cells/μL and plasma HIV-1 RNA levels >2000 copies/mL and initiated a HAART regimen of lopinavir/ritonavir (400 mg/100 mg twice daily), stavudine (40 mg for persons weighing ≥60 kg and 30 mg for persons weighing <60 kg, twice daily), and emtricitabine (200 mg daily). A sample size of 90 subjects was estimated to ensure an 80% probability of detecting an 80-cell difference in numbers of naive CD4+ cells between the age groups after 48 weeks. The first 56 (28 each of older and younger) subjects also participated in a detailed immunologic substudy that included measurements of plasma cytokines, TREC DNA, and radiographic estimates of thymic volume. Forty-eight age-matched, HIV-uninfected healthy volunteers were included for baseline comparisons. Additional eligibility criteria for participation in the study have been described elsewhere [31].

Toxicities were graded by use of the standardized Adult AIDS Clinical Trials Group (AACTG) toxicity grading criteria. Participants who changed medications because of toxicities or adverse events or who had virologic failure (HIV-1 RNA levels >200 copies/mL after 24 weeks) could remain in the study if a medication regimen compatible with contemporary HAART guidelines was continued [33]. A virologic relapse was defined as ≥2 consecutive plasma HIV-1 RNA measurements >200 copies/mL after at least 2 measurements ≤200 copies/mL. Institutional review boards at each site approved the study, and all participants provided written, informed consent.

Virologic and immunologic studies. Plasma HIV-1 RNA levels were measured by use of quantitative HIV-1 RNA polymerase chain reaction (PCR) assays (Amplicor HIV-1 MONITOR version 1.0; Roche Molecular Systems) with a sensitivity of 50 copies/mL. Lymphocyte subsets were enumerated in fresh whole blood labeled with murine monoclonal antibodies against CD3, CD4, CD8, CD16, CD19, CD28, CD38, CD56, CD95, CD45RA, CD45RO, CD62L, and HLA-DR (Pharmingen) by 3-color flow cytometry by use of an AACTG consensus immunologic protocol [34]. Naive cells were defined as CD45RA+/CD62L+, memory cells were defined as CD45RO+/CD45RA−, NK cells were defined as CD3+/CD56+ and/or CD16+, and B cells were defined as CD19+. Absolute lymphocyte counts were derived from complete blood differential counts. CXCR4 and CCR5 receptor densities on CD4+ and CD14+ cells were determined by immunofluorescence intensity according to AACTG consensus methods [34]. Spontaneous apoptosis was measured in fresh peripheral blood mononuclear cells (PBMCs) by use of propidium iodide staining, as described elsewhere [35].

Thymic volumes were estimated from noncontrasted chest computed-tomography scans at baseline and week 48 and were scored as 0–5 (0, no visible thymic tissue [i.e., entirely replaced by fat]; 1, minimal, barely recognizable soft tissue; 2, minimal but more obvious soft tissue; 3, moderate thymic tissue; 4, moderate soft tissue but to a greater extent; and 5, thymic mass of concern for thymoma) [19]. Thymic scores were assigned by agreement between 2 readers (B.H.G. and I.R.F.) who were blinded to the age groups of the subjects.

Genomic DNA was extracted from cryopreserved PBMCs by use of DNAzol (GIBCO BRL). Signal joint (sj) TREC DNA was quantified by real-time PCR and is reported as the number of TREC copies per microgram of total genomic PBMC DNA, by use of specific primers for sjTRECs, as described elsewhere [36].
Plasma specimens were collected in EDTA and stored at or below \(-70^\circ\text{C}\). Cytokines and cytokine receptors were measured in thawed plasma aliquots by use of commercial ELISA kits (soluble tumor necrosis factor receptor II [sTNFR-II], soluble IL-2 receptor [sIL-2R; Biosource], and IL-7 [QuantikineHS]), in accordance with the manufacturer’s specifications. The lower limits of quantification were 0.1, 12, and 100 pg/mL for IL-7, sIL2R, and sTNFR-II, respectively.

**Statistical analysis.** Analyses were performed by use of SAS (version 8.2; SAS Institute) and S-Plus software (version 3.4; Mathsoft). Age-group differences of ordinal and continuous variables were compared by use of the Wilcoxon rank sum test; binary outcomes were compared by use of Fisher’s exact test. A sign test was used to test whether median changes from baseline within each group differed from 0. Statistical significance required 2-tailed \(P<.05\), and there were no adjustments for multiple testing.

Multivariable linear-regression models for changes in naive CD4+ and CD8+ and total CD4+ cell counts included candidate independent variables categorized as markers of viral replication (quantitative plasma HIV-1 RNA level and viral load suppression to \(<50\) copies/mL as a binary variable), immune activation (sTNFR-II plasma levels, percentage of spontaneous lymphocyte apoptosis, and percentage of CD8+/HLA-DR+/CD38+ cells), markers of thymic function (TREC DNA content and thymic score), plasma IL-7 levels, and age (as a continuous and a binary variable); these were derived from data on subjects in the immunologic substudy only. Model selection took into account each of these categories; contributions of variables within each category were assessed in the order of the greatest univariate correlation with the outcome variable. Potential interactions were explored between thymic function markers and candidate variables. Significance was determined by use of a partial \(F\) test and was defined as \(P<.05\). Listwise deletions were applied to address missing data. Selected models were used only when exclusion of the most extreme outlier, as determined by Cook’s distance, did not substantially change the results. Interactions identified by these models were illustrated by use of Spearman rank correlations that were stratified, when indicated, according to the median values of thymic scores at baseline and week 48 and to baseline plasma IL-7 levels.

**RESULTS**

**Attrition, toxicity, and virologic responses to study drugs.** Ninety-two subjects—46 older (median age, 50 years; range, 45–79 years) and 46 younger (median age, 26 years; range, 18–30 years) persons—were enrolled in 26 AACTG sites between October 2000 and August 2001. Eighty subjects (87%) remained in the study through week 48. Two subjects were excluded from the analysis; 1 did not fulfill the eligibility criteria, and 1 was lost to follow-up before initiating the study medication.

There were no differences in attrition between the age groups, with 4 older and 6 younger subjects having withdrawn from the study by week 48 (\(P = .74\)). Older subjects had similar numbers of serious laboratory toxicity episodes (12 grade \(\geq 3\) episodes in 11 older subjects vs. 13 grade \(\geq 3\) episodes in 11 younger subjects). Thirty older subjects (66.7%) and 26 younger subjects (57.7%) achieved viral suppression to \(<50\) HIV-1 RNA copies/mL by week 48 (\(P = .51\)), whereas 7 younger and 0 older subjects had a virologic relapse during this time (\(P = .012\)).

**Age-associated differences in immune markers.** Smaller 48-week median increases from baseline levels in naive CD4+ cells (48 vs. 85 cells/\(\mu\)L; \(P = .047\)) and in the percentage of CD8+ cells that were naive (9% vs. 13%; \(P = .032\)) were evident in older, compared with younger, subjects (figure 1A and 1B). Older subjects also had smaller increases in naive CD8+ cells (56 vs. 102 cells/\(\mu\)L; \(P = .18\)) and total CD4+ cells (155 vs. 188 cells/\(\mu\)L; \(P = .72\)) and greater increases in total CD8+ cells (22 vs. 0 cells/\(\mu\)L; \(P = .48\)), but these differences were not significant. Thus, naive T cell regeneration was impaired in older subjects.

There were no significant age-associated differences in the changes of lymphocyte activation markers, including HLA-DR/CD38 and CD95 (Fas), or in the coreceptor for T cell activation CD28 on either CD4+ or CD8+ cells, nor were there any differences in the changes in B cells or NK cell counts or in the changes of CCR5 or CXCR4 chemokine receptor densities on either CD4+ cells or CD14+ monocytes according to age (data not shown).

Median thymic scores decreased in both age groups (figure 1D). Although there were no significant differences between age groups in these changes, when the changes were examined within each age group, the 48-week reductions in thymic score from baseline levels were significant in older subjects (\(P = .039\)) but not in younger subjects (\(P = .57\)). Despite exhibiting smaller median increases in naive T cells, older subjects also had greater increases in TREC content (2.92 vs. 1.07 log10 TREC copies/\(\mu\)g DNA; \(P = .031\)) (figure 1C).

In addition to previously reported baseline differences [31], plasma sTNFR-II levels were higher in HIV-1–infected subjects than in healthy control subjects and in older, compared with younger, HIV-1–infected subjects (table 1). Reductions in sTNFR-II levels after 48 weeks were similar between the age groups. Baseline plasma IL-7 levels were higher in HIV-1–infected subjects than in control subjects, but these differences were significant only in younger subjects. There were no significant age-associated differences in plasma IL-7 levels or in their 48-week changes from baseline. Reductions in spontaneous lymphocyte apoptosis also were not significantly different between the age groups (data not shown).

**Multivariable linear-regression models and correlations with thymic markers.** Thymic score independently contributed to multivariable linear-regression models of first-phase
Figure 1. Naive CD4+ cell counts (A), naive CD8+ cell percentages (B), T cell receptor excision circle (TREC) DNA in peripheral blood mononuclear cells (C), and thymic scores (D) in older (Old) and younger (Yng) HIV-1–infected subjects at baseline and after 48 weeks of highly active antiretroviral therapy, compared with those in healthy, age-matched control subjects (Ctl). The median values (Med) and sample sizes (n) are described for each subject group.

Changes in naive and total CD4+ cell counts and to models of overall changes in naive CD4+ cell counts (figure 2A and 2C and table 2). In addition to these main effects, significant interactions involving thymic score with baseline plasma IL-7 levels were identified in models of first-phase changes in naive and total CD4+ cell counts in response to HAART (table 2). These interactions, when illustrated by stratified Spearman rank correlations, demonstrated a correlation between IL-7 levels and first-phase naive and total CD4+ cell regeneration in subjects with low but not high thymic scores (figure 3A and 3B). Models of second-phase (naive CD4+, CD8+, and total CD4+) changes in T cell counts (not shown) also indicated that
Table 1. Baseline and 48-week changes from baseline in plasma cytokine levels in older and younger HIV-1–infected and healthy control subjects.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>HIV-1–infected subjects</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>48-week changes from baseline</td>
</tr>
<tr>
<td></td>
<td>Older</td>
<td>Younger</td>
</tr>
<tr>
<td>STNFR-II, pg/mL</td>
<td>9.70</td>
<td>6.94</td>
</tr>
<tr>
<td>sIL-2R, ng/mL</td>
<td>288.4</td>
<td>219.1</td>
</tr>
<tr>
<td>IL-7, pg/mL</td>
<td>7.41</td>
<td>6.06</td>
</tr>
</tbody>
</table>

NOTE. IL, interleukin; sTNFR, soluble tumor necrosis factor receptor.

a $P < .001$ for HIV-1–infected subjects at baseline vs. their corresponding age-matched healthy control subjects; $P = .014$ for older vs. younger HIV-1–infected subjects.

b $P = .023$ for younger HIV-1–infected subjects at baseline vs. younger control subjects.

the association of IL-7 levels with these outcomes was significantly different in subjects with low versus high thymic scores at week 48 ($P = .006$, $P = .01$, and $P = .005$, respectively). In these models, correlations between changes in IL-7 at 48 weeks and second-phase changes in T cell counts were evident in subjects with low thymic scores who also had high baseline IL-7 levels, which suggests that therapy-associated reductions in IL-7 may be associated with attenuated second-phase increases in T cells (table 2 and figure 3E). No significant models for second-phase changes in T cell change counts were identified in subjects with high thymic scores (figure 3F).

Additional interactions between baseline thymic scores and first-phase changes in immune activation (measured by the percentage of CD8+/HLA-DR+/CD38+ cells) were evident in models of first-phase changes in naive CD4+ and CD8+ cells, wherein correlations between these increases in T cells and reductions in immune activation were evident in subjects with high but not low baseline thymic scores (table 2 and figure 3C and 3D). Overall reductions in immune activation also were associated with overall naive and total CD4+ cell increases, but interactions involving thymic score were not identified in these models (table 2).

Age was negatively correlated with baseline thymic score ($r = −0.49$, $P < .001$), but variability in age was evident within each thymic score category (subjects with low baseline thymic scores: median age, 44 years; range, 20–79 years and subjects with high baseline thymic score: median age, 28 years; range, 19–49 years). Age independently contributed only to models for first-phase changes in total CD4+ cell counts (table 2). Subjects with low thymic scores had smaller increases in median naive CD4+ cells (57 vs. 111 cells/μL; $P = .04$), smaller increases in proportional naive CD8+ cells (9% vs. 14.5%; $P = .26$), and a higher TREC content at week 48 (2.04 vs. 1.15 log10 copies/μg of total DNA; $P = .23$). Thus, the age-associated differences that we observed were similar when compared according to baseline thymic score categories.

At baseline, naive T cells were correlated with thymic score and with TREC content (table 3). Despite overall reductions

Figure 2. First-phase (weeks 0–12) changes in naive CD4+ cell counts vs. baseline thymic score (A); second-phase (weeks 12–48) changes in naive CD4+ cell counts vs. overall changes in thymic score (B); and overall changes in naive CD4+ cell counts (weeks 0–48) vs. baseline thymic score (C) with Spearman rank correlations and $P$ values; $P = .028$ for the contribution of baseline thymic score to multivariable linear-regression model of overall changes in naive CD4+ cell counts.
Table 2. Models of T cell changes.

<table>
<thead>
<tr>
<th>Phase and cell type, measurement</th>
<th>$R^2$</th>
<th>Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-phase naive CD4+ cells</td>
<td>0.48</td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Week-0–12 % CD8/HLA-DR/CD38</td>
<td>0.473</td>
<td>0.554</td>
<td></td>
</tr>
<tr>
<td>Week-0 IL-7 level</td>
<td>4.184</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score</td>
<td>41.937</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score × week-0–12 % CD8/HLA-DR/CD38</td>
<td>-2.447</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score × week-0 IL-7 level</td>
<td>-6.431</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>First-phase naive CD8+ cells</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week-0–12 % CD8/HLA-DR/CD38</td>
<td>3.675</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score</td>
<td>30.014</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score × week-0–12 % CD8/HLA-DR/CD38</td>
<td>-6.604</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>First-phase total CD4+ cells</td>
<td>0.43</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td>75.550</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Week-0 log_{10} HIV-RNA</td>
<td>46.101</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>Week-0 IL-7 level</td>
<td>5.797</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score</td>
<td>268.434</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score × week-0 IL-7 level</td>
<td>-16.461</td>
<td>0.002</td>
<td></td>
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<tr>
<td>Second-phase naive CD4+ cells</td>
<td>0.67</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Week-0–48 IL-7 level</td>
<td>-8.748</td>
<td>0.011</td>
<td></td>
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<tr>
<td>Week-0 IL-7 level</td>
<td>2.390</td>
<td>0.175</td>
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</tr>
<tr>
<td>Week-0–48 IL-7 level × week-0 IL-7 level</td>
<td>0.8644</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Second-phase naive CD8+ cells</td>
<td>0.51</td>
<td>0.009</td>
<td></td>
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<tr>
<td>Week-0–48 IL-7 level</td>
<td>-15.875</td>
<td>0.015</td>
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<tr>
<td>Week-0 IL-7 level</td>
<td>0.8883</td>
<td>0.76</td>
<td></td>
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<tr>
<td>Week-0–48 IL-7 level × week-0 IL-7 level</td>
<td>1.1985</td>
<td>0.006</td>
<td></td>
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<tr>
<td>Second-phase total CD4+ cells</td>
<td>0.48</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>Week-0–48 IL-7 level</td>
<td>-20.0042</td>
<td>0.078</td>
<td></td>
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<tr>
<td>Week-0 IL-7 level</td>
<td>16.9041</td>
<td>0.011</td>
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<tr>
<td>Week-0–48 IL-7 level × week-0 IL-7 level</td>
<td>2.4070</td>
<td>0.003</td>
<td></td>
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<tr>
<td>Overall naive CD4+ cells</td>
<td>0.24</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Week-0–48 % CD8/HLA-DR/CD38</td>
<td>-1.8819</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Week-0 thymic score</td>
<td>48.8153</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Overall total CD4+ cells</td>
<td>0.08</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Week-0–48 % CD8/HLA-DR/CD38</td>
<td>-2.6412</td>
<td>0.011</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Independent factors that were associated with these changes in T cell counts are listed with their regression coefficients and P values. Significant interactions between factors are given in bold type. To better understand the 3-way interactions that were identified in the second-phase models, separate models are summarized in subjects with only low thymic scores. No models were identified for overall naive CD8+ cells. IL, interleukin.

in thymic score, second-phase increases in naive T cells were correlated with increases in thymic score (naive CD4+ cells, $r = 0.32, P = .038$; naive CD8+ cells, $r = 0.30, P = .054$) (figure 2B), but changes in naive T cell counts were not correlated with changes in TREC content. In an additional model, reductions in spontaneous lymphocyte apoptosis were associated with reductions in immune activation (48-week reductions in the percentage of CD8+/HLA-DR+/CD38+ cells) when adjusted by baseline TREC content ($P = .043$), which suggests a possible role of activation-associated apoptosis in association with the changes in HLA-DR/CD38 expression.

**DISCUSSION**

The reasons for poorer outcomes of older HIV-1–infected persons remain incompletely understood. In the present study, attrition and serious toxicities with therapy were similar between age groups. Although initial rates of viral suppression were also comparable, younger subjects had higher rates of viral rebound. These data suggest that older subjects may have better adherence to treatment regimens and are consistent with previous demonstrations of the safety and efficacy of HAART in older persons [37–41].

Older subjects also had lower levels of naive T cell regeneration, despite similar rates of viral suppression and comparable reductions in markers of immune activation. Given the disproportionate defects in naive T cell homeostasis that is associated with HIV-1 disease, it is plausible that these age-associated differences in naive T cell potential, which have also been described by others [15, 16], may contribute to the pathogenesis of age-associated accelerated disease progression.
Figure 3. Spearman rank correlations between first-phase changes in naive CD4+ cell counts and baseline plasma interleukin (IL)-7 levels (A and B) and between first-phase changes in naive CD4+ cell counts and percentages of CD8+/HLA-DR+/CD38+ cells (C and D), stratified by the median value of baseline thymic scores (low thymic scores, ≤2; high thymic scores, >2). Second-phase changes in naive CD4+ cell counts vs. overall changes in plasma IL-7 levels (E and F) were stratified by the median value of week-48 thymic scores (low thymic scores, ≤1; high thymic scores, >1); black circles represent subjects with high baseline plasma IL-7 levels (greater than the median, 6.87 pg/mL), and white circles represent subjects with low baseline plasma levels of IL-7 (≤6.87 pg/mL). *The correlation is for black circles (subjects with high baseline IL-7 levels) only.

Multivariable linear-regression models of changes in T cell counts, to identify factors associated with these differences in naive T cells, identified significant interactions between thymic score, a radiographic estimate of thymic volume, and IL-7 (in models of first-phase changes in naive and total CD4+ cell counts and of second-phase changes in naive CD4+, CD8+, and total CD4+ cell counts) and between thymic score and reductions in immune activation (as measured by the percentage of CD8+/HLA-DR+/CD38+ cells in models of first-phase changes in naive CD4+ and CD8+ cell counts). The differential
relationships that are implied by these interactions suggest distinct mechanisms of T cell regeneration, according to this thymic function marker.

Evidence for thymic-independent peripheral expansion has been demonstrated in lymphocyte-depleting conditions—including HIV-1 disease, bone-marrow transplantation—and in normal aging [26, 27, 42, 43]. A central role of IL-7 in this process has been demonstrated in animal models, where IL-7 improved T cell survival and enhanced cellular proliferation [44–47]. In HIV-1 disease, IL-7 levels are inversely correlated with CD4+ cell counts and decrease in response to therapy [48–50]. We provide additional evidence for an important role of IL-7 in thymic-independent T cell regeneration, wherein correlations between changes in T cell counts and plasma IL-7 levels, or changes in these levels, were evident only in subjects with low thymic scores.

CD4+ cell increases have been variably demonstrated in association with reductions in HLA-DR/CD38 expression on CD4+ or CD8+ cells [51–53]. Consistent with the proposed importance of immune activation in early lymphocyte redistribution [54–56], significant correlations between first-phase increases in naive T cells and reductions in this immune activation marker on CD8+ cells were evident but only in subjects with high thymic scores. Also consistent with the association between HLA-DR expression and activation-induced apoptosis in lymphocytes of adults after chemotherapy for cancer [57], the relationship between immune activation and spontaneous apoptosis supports a role of activation-induced apoptosis in this process [3, 4, 58].

We estimated thymic function using thymic score and the sjTREC DNA content in PBMCs and their changes. As expected, age was inversely correlated with thymic score and TREC content at baseline. Reductions in thymic score in response to therapy were significant only in older subjects; however, in them, greater increases in TREC content were unexpectedly observed. Although correlations between naive T cell counts and their changes in response to therapy have been demonstrated in association with radiographically estimated thymic volumes [19–21, 23, 24], such estimates of thymic volume cannot distinguish between thymopoietically active epithelial tissue and inactive peripheral vascular space, which accumulates with age and functions as peripheral lymphatic tissue [59]. Hence, estimated thymic volumes may not strictly correlate with thymic function. Accordingly, although the reductions in thymic score that we observed may represent a homeostatic response by the thymus to T cell repletion [19], these reductions may also merely reflect lymphocyte redistribution from perivascular thymic tissue, particularly given that these reductions were significant only in older subjects, in whom a greater accumulation of perivascular tissue would be expected.

sjTRECs are episomal DNA products of T cell receptor α-chain gene rearrangement that occurs within double-positive (CD3+CD4+CD8+) thymocytes, and their frequency has been linked to thymic output [17]. As with thymic score, the interpretation of thymic output using this marker also difficult. Because they do not replicate, TRECs are diluted by cell division, so therapy-related changes in the proliferation of TREC-bearing cells or changes in their longevity can influence TREC frequencies [56], as can the preferential early redistribution of such cells from lymphoid tissue to the circulation [60, 61]. Differences in TREC content within CD4+ and CD8+ cells further complicate the interpretation of this marker when it is measured in unseparated PBMCs [62]. Recently, using a calculated ratio of signal joint:DNA TRECs that reflected thymocyte proliferation but was not affected by peripheral cell division, Dion et al. [25] found that intrathymic proliferation was impaired in HIV infection and could be improved in some patients in response to antiretroviral therapy.

The reasons for the greater TREC increases in older subjects in the present study are not apparent, but they may be multifactorial. Conceivably, they may have resulted from a greater redistribution of TREC-bearing lymphocytes in older subjects that is perhaps reflected by the significant reduction in thymic score that also was evident in these subjects. Alternatively, the improved survival of TREC-bearing cells in the circulation (perhaps mediated by IL-7) or the reduced proliferation of lym-

Table 3. Spearman rank correlations (and P values) between baseline naive T cells and their overall (weeks 0–48) and second-phase (weeks 12–48) changes in level, with thymic score and T cell recipient excision circle (TREC) content at baseline and with overall changes in thymic score and TREC content.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Week 0 naive CD4+</th>
<th>Week 0 naive CD8+</th>
<th>Week-0 naive CD8+</th>
<th>Week-0 naive CD4+</th>
<th>Week-12–48 naive CD8+</th>
<th>Week-12–48 naive CD4+</th>
<th>Week-0 naive CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement</td>
<td>Thymic score</td>
<td>TREC content</td>
<td>Thymic score</td>
<td>TREC content</td>
<td>Thymic score</td>
<td>TREC content</td>
<td>TREC content</td>
</tr>
<tr>
<td>Week 0 naive CD4+</td>
<td>0.31 (.024)</td>
<td>0.32 (.018)</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0 naive CD8+</td>
<td>0.23 (.093)</td>
<td>0.28 (.043)</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week-0 naive CD4+</td>
<td>0.26 (.074)</td>
<td>-0.04 (.818)</td>
<td>0.06 (.710)</td>
<td>-0.11 (.493)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week-0 naive CD8+</td>
<td>0.13 (.375)</td>
<td>-0.11 (.458)</td>
<td>0.19 (.247)</td>
<td>0.13 (.428)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week-12–48 naive CD8+</td>
<td>0.03 (.848)</td>
<td>-0.08 (.616)</td>
<td>0.32 (.038)</td>
<td>0.06 (.724)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week-12–48 naive CD4+</td>
<td>0.12 (.428)</td>
<td>-0.21 (.198)</td>
<td>0.30 (.054)</td>
<td>0.19 (.258)</td>
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<td></td>
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</tr>
</tbody>
</table>
phocytes in older, compared with younger, subjects may have contributed to these age-associated differences in TREC content. In support of the latter possibility, reductions of the replication capacities of both naive and memory CD4+ cells of older persons are suggested by the age-related shortening of their telomeres [63].

Despite the complexities in interpreting thymic output by use of TREC content and thymic score, the consistent interactions involving thymic score that were present in models of changes in T cell counts suggest distinct pathophysiologic mechanisms that may contribute differentially to HAART-induced cellular restoration in persons with smaller and larger estimated thymic volumes. As has been seen in animal models, cellular restoration in subjects with low thymic scores may rely primarily on IL-7-mediated, thymic-independent mechanisms [50]. Conceivably, lymphocytes expanded in this way may also be less susceptible to activation-induced cell death resulting from the effects of IL-7 on Bcl-2 up-regulation [44].

**AIDS CLINICAL TRIALS GROUP 5015 AND 5113 STUDY TEAM MEMBERS**

The following investigators and institutions participated in the studies. Scott C. Brun, Kevin W. Garren, and Bruce G. Richards (Abbott Laboratories, Abbott Park, IL); Helen Fitch (Beth Israel Deaconess Medical Center, Boston, MA); Jeffrey Olson and Kristy Porter (Bristol Myers-Squibb, New York, NY); Jane Baum, Kim Whately, and Ronald Johnson (Case Western Reserve University, Cleveland, OH); Jennifer Janik and Nancy Webb (Frontier Science and Technology Research Foundation, Amherst, NY); Miriam Chernoff (Harvard School of Public Health, Boston, MA); James Richardson, Greta Clement, and Michael P. Dube (Indiana University, Bloomington); Charles Flexner and Albert Wu (Johns Hopkins University, Baltimore, MD); Paul Fidel (Louisiana State University, Baton Rouge); Amy Sbrolla (Massachusetts General Hospital, Boston); Mary Sarah Dolan (Mount Sinai Medical Center, New York, NY); Susan Cu-Uvin, Joan Gormley, and Karen T. Tashima (Miriam Hospital, Providence, RI); Stanley Slater (National Institute on Aging, Bethesda, MD); Dan Longo and Dennis Taub (National Institute on Aging, Baltimore, MD); Robert L. Murphy (Northwestern University, Evanston, IL); Susan L. Koletar and Laura Laughlan (Ohio State University, Columbus); MaryAnn Gianesin, Ruth Davis, Elke Narkiewicz, and John Voris (Rush University Medical Center, Chicago, IL); Kareann Makhuli and Frank Rouseau (Gilead Sciences, Durham, NC); David Shugars (University of Colorado, Denver); Nancy Mantz (University of Pittsburgh, Pittsburgh, PA); Alexandra Nesbit and Jackie Kaufman (University of North Carolina, Chapel Hill); Jose G. Castro and Roberto A. Monroig (University of Miami, Coral Gables, FL); Ian Frank and Wayne Wagner (University of Pennsylvania, Philadelphia); Connie A. Funk and Sona Avedian (University of Southern California, Los Angeles); Philip Keiser and Tianna Petersen (University of Texas, Southwest, Galveston); Joanne Stekler and Shelia Dunaway (University of Washington, Seattle); and GeYoul Kim and Eric Lawrence (Washington University, St. Louis, MO).

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**References**


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