Incomplete Reconstitution of T Cell Subsets on Combination Antiretroviral Therapy in the AIDS Clinical Trials Group Protocol 384

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(See the editorial commentary by Geng and Deeks on pages 362–4)

Background. Initiation of combination antiretroviral therapy (ART) results in higher total CD4 cell counts, a surrogate for immune reconstitution. Whether the baseline CD4 cell count affects reconstitution of immune cell subsets has not been well characterized.

Methods. Using data from 978 patients (621 with comprehensive immunological assessments) from the AIDS Clinical Trials Group protocol 384, a randomized trial of initial ART, we compared reconstitution of CD4⁺, CD4⁺ naive and memory, CD4⁺ activation, CD8⁺, CD8⁺ activation, B, and natural killer cells among patients in different baseline CD4⁺ strata. Reference ranges for T cell populations in control patients negative for human immunodeficiency virus (HIV) infection were calculated using data from AIDS Clinical Trials Group protocol A5113.

Results. Patients in the lower baseline CD4⁺ strata did not achieve total CD4⁺ cell counts similar to those of patients in the higher strata during 144 weeks of ART, although CD4⁺ cell count increases were similar. Ratios of CD4⁺ naive-memory cell counts and CD4⁺:CD8⁺ cell counts remained significantly reduced in patients with lower baseline CD4⁺ cell counts (≤350 cells/mm³). These immune imbalances were most notable for those initiating ART with a baseline CD4⁺ cell count ≤200 cells/mm³, even after adjustment for baseline plasma HIV RNA levels.

Conclusions. After nearly 3 years of ART, T cell subsets in patients with baseline CD4⁺ cell counts >350 cells/mm³ achieved or approached the reference range those of control individuals without HIV infection. In contrast, patients who began ART with ≤350 CD4⁺ cells/mm³ generally did not regain normal CD4⁺ naive-memory cell ratios. These results support current guidelines to start ART at a threshold of 350 cells/mm³ and suggest that there may be immunological benefits associated with initiating therapy at even higher CD4⁺ cell counts.

For nearly 25 years, CD4⁺ cell counts have been used as the primary indicator of HIV-1 disease progression and when to start antiretroviral therapy (ART) [1, 2]. Initiating antiretrovirals in patients with higher CD4⁺ cell counts may result in higher total CD4⁺ cell counts and more-durable virological suppression [3–8]. There is also a survival benefit associated with initiating ART before the CD4⁺ cell count decreases to <200 cells/mm³ [4, 9–11]; however, when initiated at 350–201 cells/mm³, benefits are less clear [12–14]. US and European consensus guidelines released in December 2007 recommend treating patients who have CD4⁺ counts ≤350 cells/mm³ [1, 2]. European AIDS Clinical Society guidelines also recommend initiating therapy in individuals with CD4⁺ cell counts of 350–500 cells/mm³ and elevated HIV viral loads (VLs) (i.e., >100,000 copies/mL), a rapidly decreasing CD4⁺ cell count (>50–100 cells/mm³), age >55 years, or hepatitis C virus coinfection.
Figure 1. Median (interquartile range) CD4⁺ cell counts (A), CD4⁺ naive cell counts (B), and CD4⁺ memory cell counts (C) by baseline CD4⁺ stratum and study week for patients who underwent comprehensive immunological assessments by advanced flow cytometry. The shaded band reflects the lowest and highest interquartiles of the 2 age groups of HIV-negative control subjects (from AIDS Clinical Trials Group protocol A5113) [33].
Figure 2. Median (interquartile range) activated CD4+ cell counts (CD4+/CD38+/HLA-DR+) for patients who underwent comprehensive immunological assessments by advanced flow cytometry. Percentages (A) and absolute counts (B) by baseline CD4+ stratum over time are shown. The shaded area reflects the lowest and highest interquartiles of the 2 age groups of HIV-negative control subjects (from AIDS Clinical Trials Group protocol A5113) [33].

Whether more-complete reconstitution of immune subsets occurs when ART is initiated at higher CD4+ cell counts is not well characterized.

CD4+ cell depletion is the hallmark of HIV infection and occurs as a consequence of viral replication and resulting cytolysis and apoptosis [15]. The latter has been shown to correlate with T cell activation and expression of TNF-α [16]. CD4+ cell loss is associated with increased CD8+ cell activation and memory CD8+ cells [17], which are predictive of HIV disease progression and death [18]. ART helps to restore circulating T cells by decreasing cell turnover, redistributing T cells, and increasing thymic output [19, 20]. Immunological reconstitution is typically measured by circulating CD4+ cell counts, which follow a biphasic pattern: an initial rapid increase during the first few months of ART, followed by a slower increase [21–25]. Among individuals with virological suppression, CD4+ cell counts continue to increase throughout 5 years, regardless of the baseline CD4+ cell count [26]. Several cohort studies have observed that patients who started ART with CD4+ cell counts <350 cells/mm³ were less likely to achieve normal

Figure 3. Median (interquartile range) CD8+ cell counts by baseline CD4+ stratum over time for patients who underwent comprehensive immunological assessments by advanced flow cytometry. The shaded area reflects the lowest and highest interquartiles of the 2 age groups of HIV-negative control subjects (from AIDS Clinical Trials Group protocol A5113) [33].
levels [7, 12, 27]. However, these cohort studies did not include detailed assessments of immune cell subsets, and observations based on total CD4+ cell counts alone may not accurately reflect immune reconstitution [21, 28, 29].

AIDS Clinical Trials Group (ACTG) protocol 384 was a large international trial that compared different ART strategies in treatment-naive patients. As reported elsewhere, the CD4+ cell count increase was not affected by initial treatment assignment [30]. Younger age, female sex, higher baseline CD4+ naive-memory cell ratio, higher HIV RNA level, and virological suppression after starting ART were associated with greater increases in total CD4+ cell count [31]. A subset of patients had a CD4+ cell count increase of ≤100 cells/mm³, despite long-term virological suppression, and this immune deficit correlated with persistent T cell activation. We now report differences in immune cell subsets by baseline CD4+ cell count stratum, and we compare these values with those of healthy HIV-negative patients.

METHODS

ART-naive HIV-1-infected patients were randomized to 6 initial ART strategies composed of 2 nucleosides: stavudine and didanosine, or lamivudine and zidovudine, combined with either nelfinavir, efavirenz, or both (ACTG protocol 384). After week 24, patients were required to switch to a different regimen after 2 consecutive VLs of >200 copies/mL [30, 32]. Plasma HIV-1 VLs were measured every 4 weeks until week 24, then...
Figure 6. Median (interquartile range) CD4+ naive-memory cell ratios by baseline CD4+ stratum and study week for patients who underwent comprehensive immunological assessments by advanced flow cytometry and HIV-negative control subjects (from AIDS Clinical Trials Group [ACTG] protocol A5113). The y-axis reflects the absolute count for both CD4+ naive and memory cells. CD4+ naive-memory cell ratios are shown above the bars at each time point, and the interquartile ranges are shown above, in parentheses. Results for HIV-negative control subjects from ACTG protocol A5113 are shown to the right of the week 144 bars, for comparison. After controlling for baseline HIV RNA level, the CD4+ naive-memory cell ratio for stratum 1 was significantly different from stratum 3 (weeks 0 and 24), stratum 4 (weeks 0, 24, 48, and 96), and stratum 5 (weeks 0, 24, and 48), and the stratum 2 CD4+ naive-memory ratio was significantly different from stratum 4 (weeks 24 and 96) and stratum 5 (weeks 0, 24, and 48).

Study definitions. Baseline CD4+ cell count strata 1–5 were defined as \(\leq 50\), 51–200, 201–350, 351–500, and \(>500\) cells/mm\(^3\), respectively. Virological suppression was defined as an HIV VL \(<50\) copies/mL. Immunological success was defined as a CD4+ cell count increase of \(\geq 100\) cells/mm\(^3\) over baseline [31].

Three-color flow cytometry was performed using fresh cells, according to the ACTG protocol. Immune subsets were defined using the following markers: naive CD4+ cells defined by CD4+, CD45RA+, and CD62L+; memory CD4+ cells defined by CD4+, CD45RO+, and CD45RA-; B cells defined by CD3- and CD19+; activated CD4+ and CD8+ cells defined by CD3+ or CD8+ plus CD38+ and HLA-DR+; and natural killer cells defined by CD3-, CD56+, and/or CD16+.

Reference ranges for immune cell subsets were calculated using data from ACTG protocol A5113, which studied 48 healthy HIV-negative patients, one-half of whom were aged 18–30 years and one-half of whom were aged \(\geq 45\) years [33]. The lowest of the first quartiles and the highest of the third quartiles for the 2 age groups were used for comparison; interquartile ranges (25th–75th percentiles) appear as shaded bands on figures 1–5 and above the HIV-negative bars in figures 6 and 7.

Statistical analyses. Unless otherwise stated, analyses were based on all available data, regardless of whether patients were virally suppressed. The nominal level of statistical significance used for these exploratory analyses was .05. Tests were 2-sided and were not adjusted for multiple testing. Median and inter-

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Every 8 weeks (Roche Amplicor, version 1.0). Log\(_10\) VL values were used for all analyses; 50 copies/mL was imputed for values under the lower limit. CD4+ and CD8+ cell counts were measured at pre-entry, study entry (day 0), every 8 weeks for 48 weeks, and then every 16 weeks. A subset of patients followed at US sites with specialized flow cytometry capacity underwent comprehensive immunological assessments (naive and memory CD4+, activated CD4+ and CD8+, natural killer, and B cell measurements) at baseline and then at 24-week intervals.
Figure 7. Median (interquartile range) CD4⁺:CD8⁺ cell ratios by baseline CD4⁺ stratum over time for patients who underwent comprehensive immunological assessments by advanced flow cytometry and for HIV-negative control subjects (from AIDS Clinical Trials Group [ACTG] protocol A5113) [33]. The y-axis reflects the absolute count for both CD4⁺ and CD8⁺ cells. Median CD4⁺:CD8⁺ cell ratios are shown above the box plot at each time point, and interquartile ranges are above in parentheses. Results for HIV-negative control subjects from ACTG protocol A5113 are shown to the right of the week 144 bars, for comparison.

RESULTS

Baseline demographic characteristics. Demographic characteristics of ACTG protocol 384 have been described elsewhere [30–32]. Comprehensive immunological assessments were performed for 623 patients (64% of the study cohort). Two patients did not have baseline CD4⁺ cell counts available and were excluded. Baseline characteristics for the remaining 621 patients were similar to those of patients involved in the main study, except for race/ethnicity (table 1) [31].

Study follow-up. The percentages of patients CD4⁺ cell counts were 89%, 83%, 74%, and 61% at weeks 24, 48, 96, and 144, respectively (no statistically significant differences in follow-up by baseline stratum). There were also no differences in additional flow cytometry assessments (84%, 79%, and 73%...
Table 1. Baseline characteristics of patients with additional flow cytometry assessment, by baseline CD4+ stratum.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline CD4+ stratum (CD4+ cells/mm³)</th>
<th>All (n = 621)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (0–50) (n = 110)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (51–200) (n = 119)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (201–350) (n = 152)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (351–500) (n = 124)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (≥500) (n = 116)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>White</td>
<td>47</td>
<td>56</td>
</tr>
<tr>
<td>Hispanic</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Black</td>
<td>50</td>
<td>41</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>IDU</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td>38 (32–45)</td>
<td>37 (31–42)</td>
</tr>
<tr>
<td>HIV RNA log₁₀, median copies/mL, (IQR)</td>
<td>5.65 (5.26–6.08)</td>
<td>5.38 (4.89–5.86)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of patients, unless otherwise indicated. Race and ethnicity were self-identified according to the categories. Hispanic ethnicity was reported regardless of race. There was a smaller Hispanic proportion in patients with additional assessment by flow cytometry than those without [31]. IDU, history of or current injection drug use; IQR, interquartile range.

* By Cochran-Armitage trend test.
* By Jonckheere-Terpstra test.
* By Fisher’s exact test.

Table 2. Median CD4+ and ∆CD4+ cell counts by baseline CD4+ stratum and study week.

<table>
<thead>
<tr>
<th>CD4+ cell count assessment</th>
<th>Baseline CD4+ cell count stratum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n = 175)</td>
</tr>
<tr>
<td>Baseline</td>
<td>Median CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td>Week 24</td>
<td>Percentage of patients with CD4+ cell count</td>
</tr>
<tr>
<td></td>
<td>Median CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td></td>
<td>Median ∆CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td>Week 48</td>
<td>Percentage of patients with CD4+ cell count</td>
</tr>
<tr>
<td></td>
<td>Median CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td></td>
<td>Median ∆CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td>Week 96</td>
<td>Percentage of patients with CD4+ cell count</td>
</tr>
<tr>
<td></td>
<td>Median CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td></td>
<td>Median ∆CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td>Week 144</td>
<td>Percentage of patients with CD4+ cell count</td>
</tr>
<tr>
<td></td>
<td>Median CD4+ cell count, cells/mm³</td>
</tr>
<tr>
<td></td>
<td>Median ∆CD4+ cell count, cells/mm³</td>
</tr>
</tbody>
</table>

* Percentage of patients with CD4+ cell count measurements for that study visit in each CD4+ stratum.
* By Cochran-Armitage trend test to test trends in proportions of available CD4+ data among the CD4+ strata.
* By Jonckheere-Terpstra test.
strata ($P<.001$, by log-rank test); median, 16 weeks for strata 1 and 2 and 12 weeks for strata 3, 4, and 5. Time to virological suppression was also affected by baseline VL ($P<.001$): median, 8 weeks for patients with baseline VLs <35,000 copies/mL, 12 weeks for VLs of 35,000–100,000 copies/mL, and 16 weeks for VLs >100,000 copies/mL. In a Cox proportional hazards model controlled for age and sex, higher baseline CD4+ cell count, lower VL, and greater CD8+ activation percentage were associated with faster time to virological suppression ($P = .02$, $P < .001$, and $P = .001$, respectively).

**Lymphocytes.** At baseline, total lymphocyte counts were lower in the lower CD4+ cell count strata (medians, 690, 1102, 1144, 1710, and 2131 cells/mm$^3$ for strata 1–5, respectively; $P < .001$) and increased more than the higher strata at weeks 24, 48, 96, and 144 (all $P < .001$). However, lymphocyte counts in the lower strata remained significantly lower than in the higher strata ($P < .001$, all time points).

**CD4+ cells.** As reported elsewhere, there was a biphasic reconstitution of CD4+ cell counts: a rapid increase during the first 8 weeks followed by a more gradual increase [31]. Median and $\Delta$CD4+ cell counts (change from baseline) for baseline CD4+ strata are given in table 2. Strata did not appear to affect $\Delta$CD4+ cell count, except at week 144, partly because CD4+ cell counts for patients in the lower strata continued to increase, whereas counts for those in stratum 5 started to plateau. However, because follow-up after week 96 was limited, this finding should be interpreted cautiously. Despite these differences, patients with lower baseline CD4+ cell counts never caught up, in terms of absolute CD4+ cell counts, with those starting in higher strata. Similar findings were found for the subset of patients with additional flow cytometry (figure 1A). Among patients in stratum 4, those with a baseline VL >100,000 copies/mL, compared with $\leq$100,000 copies/mL, had significantly greater $\Delta$CD4+ cell counts at weeks 48 and 96 but not week at 144 ($P = .022$, $P = .035$, and $P = .25$, respectively) [1].

Because increases in CD4+ cell counts were similar across all strata and ART assignments, we plotted percentiles for $\Delta$CD4+ cell counts through week 144 (figure 8). With use of a CD4+ cell count increase of $\geq$100 cells/mm$^3$ as a criterion for immunologic success, a greater proportion of patients in the lower strata had immunologic success at weeks 96 and 144 ($P = .005$ and $P < .001$, respectively; table 3).

As expected, those in the lower CD4+ strata had greater increases in CD4+ percentage points after week 32. During the first 32 weeks, greater CD8+ cell count increases in the group in the lower CD4+ baseline strata negated this effect.

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**Table 3. Percentage of patients with a CD4+ cell count increase $\geq$100 cells/mm$^3$ by baseline CD4+ stratum and study week.**

<table>
<thead>
<tr>
<th>Assessment week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>54.7</td>
<td>61.1</td>
<td>67.9</td>
<td>55.2</td>
<td>55.1</td>
<td>.141</td>
</tr>
<tr>
<td>48</td>
<td>82.8</td>
<td>77.2</td>
<td>77.8</td>
<td>73.3</td>
<td>72.7</td>
<td>.063</td>
</tr>
<tr>
<td>96</td>
<td>95.2</td>
<td>93.6</td>
<td>87.5</td>
<td>84.6</td>
<td>79.0</td>
<td>.005</td>
</tr>
<tr>
<td>144</td>
<td>92.3</td>
<td>90.9</td>
<td>100.0</td>
<td>95.2</td>
<td>73.9</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* By Cochran-Armitage trend test, used on pre-entry for stratum and entry for calculating.
**CD4 naive and memory cells and activation.** CD4 naive and memory cell counts by baseline CD4 strata are shown in figure 1B and 1C. As with absolute CD4 cell counts, patients in the lower strata did not achieve the levels of those in the higher strata, and median CD4 naive and memory cell counts for patients with a baseline CD4 cell count >350 cells/mm^3 normalized by week 48, compared with HIV-negative control subjects. Naïve CD4 cell count increases were greater those in the for the higher CD4 strata (P<.001 for weeks 24, 48, and 72; P = .020 for week 96; P = .040 for week 120). Differences in memory CD4 cell counts also persisted (P<.004, for all time points).

A comparison of the CD4 naïve-memory cell ratios revealed several differences. First, the median baseline CD4 naïve-memory cell ratio for patients starting with a CD4 cell count ≤50 cells/mm^3 was 0.21 at baseline and increased to only 0.43 at week 144, failing to reach even the pretreatment median ratio for the other strata (figure 6). Second, CD4 naïve-memory cell ratios were lower among patients in lower strata (≤350 cells/mm^3), and this difference persisted through 144 weeks (P<.01, all time points). Differences in CD4 naïve-memory cell ratios by stratum were statistically significant even after adjustment for baseline VL, except at week 144. CD4 naïve-memory cell ratios for most patients in strata 1, 2, and 3 did not return to the reference range, whereas the interquartile range for strata 4 and 5 covered the median for HIV-negative patients for all time points (including baseline). In a regression model that included baseline stratum, VL, sex, and age, patients with a baseline CD4 cell count >350 cells/mm^3 had higher baseline CD4 naive-memory cell ratios (P = .008). In this model, younger age was significantly associated with a higher baseline ratio (P<.001).

Patients in the lower strata had higher activated CD4 cell percentages (figure 2A). Median activated CD4 cell percentage for stratum 1 at baseline was >40%, almost twice that of other strata. Patients in the lower strata had greater decreases in activated CD4 percentages from baseline to week 24, even after controlling for baseline VL (stratum 1 vs. 2 and stratum 2 vs. strata 3, 4, or 5; P<.001 for all). Differences in activated CD4 cell percentages became less apparent after week 24, but only patients in stratum 5 achieved levels similar to those of HIV-negative patients. In comparison of the absolute number of activated CD4 cells rather than percentages, stratum 1 was noticeably lower than other strata or HIV-negative controls at baseline (figure 2B). At week 24 and thereafter, the absolute number of activated CD4 cells for patients in all strata were similar to those of HIV-negative controls. **CD8 cell counts, CD8 activation cell counts, and CD4**:CD8 ratio. Baseline CD8 cell counts were abnormally high, except for patients in stratum 1. At week 24 and thereafter, CD8 cell counts were higher than those in HIV-negative controls (figure 3). In contrast, the activated CD8 percentage was elevated for all strata at baseline and followed a biphasic decrease (figure 4A). In comparison of absolute activated CD8 cell counts, patients in stratum 1 had lower counts at baseline and initially increased before decreasing, whereas the patients in the higher strata followed a 2-phase decrease (figure 4B). For patients in all strata, activated CD8 cell percentages and cell counts did not achieve levels similar to those of HIV-negative patients.

The median CD4**:CD8** ratios were also higher among patients in the higher strata (P<.001, for all time points; figure 7). The median CD4**:CD8** ratio in stratum 1 increased from 0.05 to 0.40 by week 144, which was still lower than the baseline of strata 4 and 5. Nevertheless, this reflects a notable 16-fold increase in CD4 cell count, which is somewhat offset by a concomitant 2-fold increase in CD8 cell count. Restricting the analysis to patients with a CD4 cell count increase >200 cells/mm^3 at weeks 48, 96, and 144 yielded similar results (P<.001 for all). The median CD4**:CD8** cell ratios for patients in all strata remained lower than for those of HIV-negative patients.

**Natural killer cells and B cells.** Natural killer cell counts slightly increased patients in strata 1–4, but counts for patients in all strata seemed normal after baseline. Baseline B cell counts tended to be slightly lower in the lower strata (figures 5A and 5B).

**DISCUSSION**

Our findings are consistent with previous descriptions of immune subset reconstitution with ART—that is, an initial phase characterized by expansion and redistribution of CD4 memory cells followed by a second phase with reconstitution of CD4 naive and memory cells and B cells and reduction of CD4 and CD8 activation [12, 16]. In this secondary analysis of ACTG protocol 384, CD4 cell count increases from baseline were similar after week 24, regardless of baseline CD4 stratum. However, CD4 cell counts for patients in the lower strata remained below those of healthy volunteers and, as in prior reports [7, 12, 33], patients in the lower strata did not achieve levels of those who started with higher CD4 cell counts, even after nearly 3 years of ART. Moreover, despite similar CD4 cell count increases across baseline CD4 strata, differences in reconstitution of immune cells subsets were noted, especially CD4**:CD8** and CD4 naive-memory cell ratios.

Patients in lower baseline strata had smaller increases in CD4 naive cells and greater increases in CD4 memory cells, which resulted in persistently abnormal absolute cell counts and CD4 naive-memory cell ratios. Interestingly, this deficit persisted even among patients who achieved a CD4 cell increase of >200 cells/mm^3. Relative differences in CD4 naive and memory cell counts suggest that a profound immunological...
deficit occurs in the lowest strata and that this deficit may not resolve despite apparently normal CD4+ cell gains with ART. Recovery of nadir CD4+ naive cell populations may be an important aspect of immune reconstitution, because lower nadir counts have been shown to correlate with suboptimal vaccine responses and blunted CD4+ cell reconstitution [31, 34, 35].

Another important finding of this analysis is the persistent T cell activation among patients in all strata. At baseline, activated CD4+ cell percentages were elevated, especially in strata 1 and 2. Total activated CD4+ cell counts, rather than percentages, were lower in patients in stratum 1, as were CD8+ and activated CD8+ cell counts, which may be explained in part by profound lymphopenia in patients in the lower strata. Reasons for persistent T cell activation despite prolonged suppressive ART are unclear, but ongoing low-level HIV replication and bacterial translocation have been implicated as potential mechanisms [36] and may be associated with higher risks of myocardial disease and cancer [37, 38].

The rapid reduction in both activated CD4+ and CD8+ cell populations coincided with the rapid first phase of immune reconstitution and control of HIV viremia, supporting the hypothesis that ART reduces inflammation and subsequent redistribution of CD4+ and CD8+ cells [16]. Baseline median CD8+ cell counts were elevated in all patients but those in the lowest stratum. The reason for lower baseline CD8+ cell counts in patients in stratum 1 is unclear, but because these quickly increased with ART, it may reflect programmed cell death 1–associated CD8+ cell exhaustion or sequestration in lymphoid tissues that can be reversed with suppression of HIV viremia [39, 40]. At week 24 and thereafter, median CD8+ cell counts remained higher for patients in all strata, compared with those in HIV-negative control subjects.

We found no differences when we restricted the analyses to patients without regimen or virological failure. This may have been due in part to the strict definition of failure used by ACTG protocol 384 and suggests that the impact of transient low-level virological failure may be different from the sustained virological failure that is more typically seen in clinical practice.

One limitation of our analysis was the relatively small number of patients who contributed week 144 data. This was expected, because ACTG 384 was designed to allow a minimum of 2 years of follow-up, but reasons for differential follow-up by strata are unclear. However, the study conclusions would be similar if only week 96 data were used.

Exploring differences among the upper strata, we were unable to establish a clear threshold at which to start ART. However, patients initiating ART with a baseline CD4+ cell count >350 cells/mm3 appeared to achieve T cell subsets more similar to those of HIV-negative volunteers (ACTG A5113), compared with most patients who started with a CD4+ count ≤350 cells/mm3, for whom “normalization” of T cell subsets was not achieved. These results suggest that relying solely on absolute CD4+ cell counts as a measure of immune reconstitution may be misleading. Understanding differences in immune cell subsets and ratios on the basis of baseline CD4+ cell count and persistent T cell activation may explain disappointing results from treatment interruption trials and higher rates of cancer [34, 37, 38, 41–43] and may refocus the goals of ART toward normalization of T cell subsets and higher CD4+ thresholds for initiating ART.

In summary, increases in CD4+ cell counts were similar for patients in all baseline strata after week 24, and those in the lower strata did not “catch up” in absolute CD4+ cell counts by week 144. Because CD4+ cell increases from baseline were similar for patients in all strata and different ART regimens, figure 8 could be used to assess patients’ CD4+ responses to ART. Total, naive, and memory CD4+ cell counts and cell ratios were lower for patients starting with a CD4+ cell count =350 cells/mm3, especially for those with a baseline CD4+ cell count ≤50 cells/mm3; these immune deficits persisted even after nearly 3 years of ART. These findings support ART initiation at a threshold of 350 cells/mm3 [1, 2] and further suggest use of an even higher CD4+ cell count, at which time CD4+ naive cell populations and naive-memory cell ratios are more likely to still be intact.

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