Determinants of CD4+ T Cell Recovery during Suppressive Antiretroviral Therapy: Association of Immune Activation, T Cell Maturation Markers, and Cellular HIV-1 DNA

Miguel Goicoechea,1 Davey M. Smith,1 Lin Liu,2 Susanne May,2 Allan R. Tenorio,3 Caroline C. Ignacio,1 Alan Landay,4,5 and Richard Haubrich1

1Department of Medicine and 2Division of Biostatistics and Bioinformatics, Department of Family and Preventive Medicine, University of California, San Diego; Departments of 3Medicine and 4Immunology and Microbiology, Rush Medical College, Chicago, Illinois

Background. Suboptimal CD4+ T cell recovery during antiretroviral therapy (ART) is a common clinical dilemma.

Methods. We analyzed viral and immunologic predictors of CD4+ T cell recovery in 116 human immunodeficiency virus type 1 (HIV-1)–infected subjects who had suppressed viremia (<50 copies/mL) while receiving ART. Successive measurements of T cell immunophenotypes and cellular HIV-1 DNA levels were obtained before and during receipt of ART. On the basis of increases in the CD4+ T cell count, subjects were classified as immunologically concordant (demonstrating an increase of ≥100 CD4+ T cells/mm3) or discordant (demonstrating an increase of <100 CD4+ T cells/mm3) after 48 weeks of ART.

Results. In adjusted analyses, CD4+ and CD8+ T cell activation at baseline was negatively associated with immunologic concordance at week 48 of ART (odds ratio [OR], 0.80 [P = .04] and 0.67 [P = .02], respectively). High memory (CDRA45/CD62L-) CD8+ T cell counts at baseline (OR, 0.33 [P < .05]) predicted less CD4+ T cell recovery, whereas increased naive CD4+ T cell counts were associated with higher increases in CD4+ T cells (OR, 1.19 [P = .052]). Neither the cell-associated HIV-1 DNA level at baseline (P = .32) nor the cell-associated HIV-1 DNA level at week 48 of ART (P = .42) was associated with immunologic concordance during ART.

Conclusions. These results support the potential clinical usefulness of the baseline determination of immune activation and maturation subsets in the prediction of CD4+ T cell recovery during viral suppression. Furthermore, identification of individuals with reduced potential for CD4+ T cell recovery during ART may provide a rationale for the initiation of early therapy for some patients.

During potent antiretroviral therapy (ART), immune recovery is characterized by suppression of HIV-1 replication and increasing CD4+ T cell counts [1]. Control of HIV-1 replication reduces CD4+ T cell loss resulting from direct cytolysis [2, 3] and may partially restore T cell homeostasis by promoting decreased T cell proliferation [4, 5], redistribution of T cells into peripheral circulation [6, 7], and improved thymic output [8]. Although many patients continue to have CD4+ T cell recovery for several years after receiving ART [9], the degree of immune recovery achieved during viral suppression is highly variable. In some individuals, increases in the CD4+ T cell count appear to plateau after the first few months of ART [10–14]. This suboptimal CD4+ T cell response during therapy, otherwise known as “immunologic discordance,” can have detrimental clinical consequences [15]. At present, there is no validated or accepted definition of immune discordance during ART. Previous investigations of immune reconstitution during ART have not always used a viral
level cutoff of \( \leq 50 \text{ copies/mL} \) to define viral suppression [10, 13, 14, 16], which has become the standard in clinical practice [17]. Therefore, the prevalence of immune discordance during ART is difficult to establish, but it may range from 6% to 30% [15, 18–20].

In general, reconstitution of CD4+ T cells during viral suppression follows a biphasic pattern [21]. During the first 3 months of ART, the number of CD4+ T cells typically increases by 50–120 cells/mm\(^3\) [10, 22, 23]. This burst is followed by a second, slower phase of T cell repopulation with an average rate of increase of 2–7 cells/mm\(^3\) per month [10, 22–24]. Immunologic discordance may result from both viral and immunologic factors. The extent of early immune recovery may be a function of prior T cell destruction, because lower CD4+ T cell nadirs have been associated with limited immune recovery during therapy [18, 25]. Furthermore, because viral replication is incompletely suppressed by ART [26], ongoing viral cytolysis may impede CD4+ T cell recovery. In addition, immune activation has been shown to be a predictor of clinical outcome and low CD4+ T cell counts, independent of the viral level [27–29]. Potentially, persistent T cell activation during viral suppression may result in continued CD4+ T cell destruction. Alternatively, individuals may have differential regenerative capacities at the initiation of ART. It has been suggested that HIV-1 infection, through a process of chronic immune activation and increased T cell differentiation, accelerates immune aging, leading to a reduction in T cell renewal and an accumulation of terminally differentiated T cells [30]. Conceivably, the degree of immune activation and the proportion of remaining naive T cells at baseline may predict subsequent immune recovery during ART.

The intent of the present study was to investigate baseline viral and immunologic predictors of immunologic concordance during potent ART in a large, well-characterized prospective cohort. Multiple determinations of immune phenotypic markers and cell-associated HIV-1 DNA were made during viral suppression (defined as \( \leq 50 \text{ copies/mL} \)), to evaluate the longitudinal change in these factors in relation to CD4+ T cell recovery.

**METHODS**

**SUBJECTS AND METHODS**

**Study population and design.** Subjects included in the present analysis were identified from a prospective, randomized, open-label trial of nelfinavir versus efavirenz plus zidovudine/lamivudine in antiretroviral-naive HIV-1–infected individuals. Criteria for enrollment in the parent study included (1) a plasma HIV-1 RNA level of \( \geq 5000 \text{ copies/mL} \), (2) a CD4+ T cell count of \( \geq 100 \text{ cells/mm}^3 \), and (3) no evidence of active opportunistic infections or severe concurrent medical conditions. Subjects were included in this analysis if they had undetectable viral levels (\( \leq 50 \text{ copies/mL} \)) at weeks 24 and 48 of ART. Immune recovery was evaluated in 2 ways: (1) as a continuous outcome evaluated by the change in the absolute CD4+ T cell count, and (2) as a categorical outcome for which subjects were considered to be immune concordant if their CD4+ T cell count increased from baseline to at least 100 CD4+ T cells/mm\(^3\) after 48 weeks of potent ART (if this increase was not noted, the subjects were considered to be discordant). Appropriate written and informed consent was obtained from all study participants.

**Viral level measurements.** The plasma HIV-1 RNA level was measured at a central laboratory by use of a quantitative HIV-1 RNA polymerase chain reaction (PCR) assay (AmpliCor HIV-1 Monitor Assay, version 1.0 [ultrasensitive method]; Roche) that has a lower limit of detection of 50 copies/mL. The cellular HIV-1 DNA level was determined in 60 eligible individuals, by randomly selecting an equal number of subjects from groups with immunologic discordance and concordance. The HIV-1 proviral DNA level was then measured in peripheral blood mononuclear cells (PBMCs) at baseline and after 48 weeks of ART. PBMCs were separated from whole venous blood by ficoll-hypaque density sedimentation and were stored at \(-150^\circ\text{C}\). Cellular HIV-1 DNA was quantified from PBMC DNA by use of a Roche PCR-based system with colorimetric detection. The primers used in this PCR-based system detect only late-stage or fully reverse-transcribed HIV-1 DNA. On the basis of genomic DNA input into PCRs, the lower limit for reliable quantification was 5 HIV-1 DNA copies/\(\mu\)g of genomic DNA, as described elsewhere [31]. Cellular HIV-1 DNA was also adjusted for the absolute CD4+ T cell count, to determine the log\(_{10}\) number of HIV-1 DNA copies/\(\mu\)g of CD4+ lymphocyte DNA.

**Immunophenotypic analysis.** Three-color flow-cytometric analysis was performed using Adult AIDS Clinical Trial Group consensus methods. In brief, EDTA-anticoagulated whole blood was obtained, and 100-\(\mu\)L aliquots were placed in 5-mL polystyrene tubes. Premixed 3-color antibody combinations were added to each tube and were incubated at room temperature for 15 min. After incubation, red blood cells were lysed, and cells were washed with PBS containing 3% fetal bovine serum. Cell samples were then fixed in 2% paraformaldehyde and were analyzed within 24 h. Antibodies for this study were all obtained from Becton Dickinson. The following antibody combinations, conjugated to fluorescein isothiocyanate phycoerythrin or peridinin chlorophyll protein, were used: CD4/CD45RO/CD45RA, CD8/CD45RO/CD45RA, CD4/CD45RA/CD62L, CD8/CD45RA/CD62L, CD4/HLA-DR/CD38, and CD8/HLA-DR/CD38. Samples were analyzed on the FACScalibur instrument with use of CellQuest Software (Becton Dickinson). Flow-cytometric assays were performed to determine the proportions of CD4+ and CD8+ lymphocytes that were naive (CD45RA+CD62L-) or activated (CD38+/HLA-DR+). Memory T cells were defined as

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Table 1. Baseline demographic and clinical characteristics of the 116 subjects who received antiretroviral therapy for 48 weeks.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Discordant(^a) subjects ((n = 48))</th>
<th>Concordant(^b) subjects ((n = 68))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), years</td>
<td>41 (33–48)</td>
<td>35 (29–41)</td>
</tr>
<tr>
<td>Sex, male, % of patients</td>
<td>79.2</td>
<td>83.8</td>
</tr>
<tr>
<td>Race/ethnicity, % of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>50</td>
<td>36.8</td>
</tr>
<tr>
<td>Black</td>
<td>39.6</td>
<td>44.1</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10.4</td>
<td>17.6</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>History of AIDS-defining conditions,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of patients</td>
<td>10</td>
<td>7.4</td>
</tr>
<tr>
<td>Efavirenz-based regimen received,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of patients</td>
<td>68.1</td>
<td>50.7</td>
</tr>
<tr>
<td>CD4(^+) T cell count, median (IQR),</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cells/mm(^3)</td>
<td>315 (215–438)</td>
<td>287 (198–402)</td>
</tr>
<tr>
<td>HIV-1 RNA level, median (IQR), log(_{10}) copies/mL</td>
<td>4.31 (4.1–4.73)</td>
<td>4.48 (4.18–4.91)</td>
</tr>
</tbody>
</table>

NOTE. IQR, interquartile range.
\(^a\) Demonstrating an increase of <100 CD4\(^+\) T cells/mm\(^3\).
\(^b\) Demonstrating an increase of >100 CD4\(^+\) T cells/mm\(^3\).
\(^c\) \(P < .01\), for comparison of subjects with immunologic discordance with subjects with concordance.

cells that do not express a naive phenotype (i.e., CD45RA\(^-\)CD62L\(^-\), CD45RA\(^+\)CD62L\(^-\), or CD45RA\(^-\)CD62L\(^+\)).

**Statistical analysis.** The association between baseline parameters and immunologic concordance at week 48 of ART was evaluated by logistic regression analyses. In addition, linear regression analyses were used to assess associations between potential factors and absolute CD4\(^+\) T cell recovery for the first study phase (baseline to week 12), the second study phase (weeks 12–48), and combined study phases (baseline to week 48). Analyses were adjusted for age, CD4\(^+\) T cell count at baseline, plasma viral level (log\(_{10}\) transformed) at baseline, and ART regimen, if these factors were significant at \(P < .05\). Sensitivity analyses were performed to assess the influence of potential outliers on model results and conclusions. Goodness of fit was assessed for multivariate models by use of the Hosmer-Lemeshow test [32]. Wilcoxon rank sum tests were used for bivariate comparisons of immune cell subsets and HIV-1 DNA content between discordant and concordant subjects. Spearman’s rank correlation test was used to evaluate the relationship between HIV-1 DNA content and different immune cell subsets. A 2-sided \(P\) value of <.05 was considered to denote statistical significance. No adjustments were made for multiple comparisons. Statistical tests were performed using the open-source statistical package R (version 1.7.0) [33].

**RESULTS**

Of the 250 subjects who were originally randomized to a treatment arm in the parent study, 88 were missing data at week 48, and 46 did not achieve viral suppression. All of the remaining 116 subjects with undetectable plasma viral levels (≤50 copies/mL) at weeks 24 and 48 were included in this analysis. Forty-eight (41%) of 116 patients had discordant CD4\(^+\) T cell responses at week 48. There was no significant difference between individuals with immune concordance and those with discordance, on the basis of sex, race/ethnicity, or history of AIDS-defining conditions. Concordant subjects were significantly younger than discordant subjects (\(P < .004\)), but both groups had similar plasma HIV-1 viral levels (\(P < .12\)) and CD4\(^+\) T cell counts (\(P < .24\)). Also, there was a trend for discordant subjects to receive an efavirenz-based regimen versus a nelfinavir-based regimen (\(P < .07\)) (table 1). The median increase in the CD4\(^+\) T cell count during the 48 weeks of ART-mediated viral suppression was 33 cells/mm\(^3\) (interquartile range [IQR], −30 to 72 cells/mm\(^3\)) and 215 cells/mm\(^3\) (IQR, 160–274 cells/mm\(^3\)) for discordant and concordant subjects, respectively.

**Association of immunophenotypic markers at baseline with immunologic concordance after 48 weeks of suppressive ART.** In unadjusted analysis, several baseline factors were associated with immunologic concordance after 48 weeks of ART. Increasing patient age significantly decreased the odds of achieving immunologic concordance (odds ratio [OR] [per 5-year increase], 0.74; \(P < .01\)), as did higher proportions of memory (CD45RA\(^-\)CD62L\(^-\)) CD8\(^+\) T cells at baseline (OR [per 10% increase], 0.41; \(P < .04\)). In contrast, a high percentage of naïve CD4\(^+\) T cells at baseline significantly increased the odds of an individual achieving immunologic concordance after 48 weeks.
High proportions of naive CD4+ T cells were associated with pressive therapy. Proportions of CD4+ or CD8+ T cells expressing T cell activation markers at baseline were not associated with CD4+ T cell recovery in univariate analysis (P = .19 and P = .11, respectively). Interestingly, other subsets of memory T cells (CD45RA+CD62L- and CD45RA-CD62L+) were not associated with CD4+ T cell recovery.

Immune markers at baseline were then evaluated in multivariate models adjusting for relevant biological and univariate predictors of concordance that were significant at P < .05 (tables 2 and 3). Consistent with analysis using proportions, higher absolute numbers of activated and memory (CD45RA-CD62L+) CD8+ T cells (P = .03 and P = .04, respectively) were significantly associated with suboptimal CD4+ T cell recovery. Similarly, there was an association between higher numbers of memory (CD45RA-CD62L+) CD4+ T cells at baseline (P = .08) and fewer increases in CD4+ T cell counts. However, naive CD8+ cells (P = .19), naive CD4+ T cells (P = .61), and activated CD4+ T cells (P = .11) were not associated with immunologic concordance when immune cell subsets were evaluated as absolute cell counts.

**CD4+ T cell recovery as a continuous outcome.** Concordant subjects had significantly greater increases in CD4+ T cells than did discordant subjects during CD4+ T cell recovery in both phase 1 (median increase, 111 vs. 28 cells/mm3; P < .001).

### Table 2. Univariate predictors of immunologic concordance after 48 weeks of successful antiretroviral therapy.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>0.74 (0.61–0.90)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Baseline value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ T cell count, cells/mm3</td>
<td>0.99 (0.98–1.00)</td>
<td>.10</td>
</tr>
<tr>
<td>HIV-1 RNA level, log10 copies/mL</td>
<td>1.79 (1.83–3.88)</td>
<td>.14</td>
</tr>
<tr>
<td>Efavirenz-based regimen received</td>
<td>0.48 (0.22–1.05)</td>
<td>.07</td>
</tr>
</tbody>
</table>

**NOTE.** A multivariate logistic model was used for this analysis. CI, confidence interval; OR, odds ratio.

### Table 3. Multivariate predictors of immunologic concordance after 48 weeks of successful antiretroviral therapy.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Percentage cell count</th>
<th>Absolute cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD38+HLA-DR</td>
<td>0.80 (0.64–0.99)</td>
<td>0.93 (0.86–1.02)</td>
</tr>
<tr>
<td>CD8+CD38+HLA-DR</td>
<td>0.67 (0.47–0.95)</td>
<td>0.98 (0.96–0.99)</td>
</tr>
<tr>
<td>CD4+CD45RA-CD62L</td>
<td>1.19 (1.00–1.41)</td>
<td>0.99 (0.97–1.02)</td>
</tr>
<tr>
<td>CD8+CD45RA-CD62L</td>
<td>1.23 (0.93–1.62)</td>
<td>0.99 (0.96–1.01)</td>
</tr>
<tr>
<td>C*D4+CD45RA-CD62L</td>
<td>0.69 (0.42–1.11)</td>
<td>0.88 (0.77–1.01)</td>
</tr>
<tr>
<td>CD8+CD45RA-CD62L</td>
<td>0.33 (0.11–0.98)</td>
<td>0.92 (0.85–0.99)</td>
</tr>
</tbody>
</table>

**NOTE.** A multivariate logistic model was used for this analysis. CI, confidence interval; OR, odds ratio.

* Odds of having an increase of >100 CD4+ T cells after 48 weeks of suppressive therapy.
* Per 5% or 5-cell/mm3 increase.
* Age, CD4+ T cell count at baseline, log10 HIV-1 RNA level at baseline, and efavirenz-based regimen are the covariates in the percentage cell count model.
* Age, log10 HIV-1 RNA level at baseline, and receipt of an efavirenz-based regimen are the covariates in the absolute cell count model.
* Per 10% or 10-cell/mm3 increase.
* Age and efavirenz-based regimen are the covariates in the absolute cell count model.

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.01) and phase 2 (median increase, 104 vs. 5 cells/mm³; \( P < .01 \)) (figure 1). None of the immune cell subsets or characteristics at baseline, including CD4⁺ T cell count at baseline, change in plasma viral level, and regimen type, were significantly associated with CD4⁺ T cell recovery in either phase 1 or phase 2 separately. Only increasing age was negatively associated with increases in the CD4 T cell count during phase 2 (\( P = .05 \)). Furthermore, when total CD4⁺ T cell recovery was analyzed as a continuous variable, despite some similar associations in reduced models, the full models failed to reproduce the associations demonstrated in logistic models that used a categorical outcome—that is, immune concordance versus immune discordance (data not shown).

**Longitudinal immunophenotypic characteristics during viral suppression.** Marked differences in both the proportion and absolute cell count of immune cell subsets were observed between discordant and concordant subjects during 48 weeks of suppressive ART (figure 2A–2F). Although pretherapy proportions of CD4⁺ cell activation were not significantly different between groups, during viral suppression, discordant subjects generally had higher levels of persistent CD4⁺ T cell activation than did concordant subjects (figure 2A). Discordant subjects also appeared to have a more senescent CD4⁺ T cell population at initiation of ART, because they had both lower baseline percentages of naive (\( P = .04 \)) CD4⁺ T cells and higher frequencies of memory (CD45RA⁺CD62L⁻) CD4⁺ T cells (\( P = .02 \)) than did concordant subjects. During viral control, these differences persisted, and discordant subjects had lower proportions of naive CD4⁺ T cells and generally higher proportions of memory (CD45RA⁺CD62L⁻) CD4⁺ T cells at weeks 12, 24, and 48 (figure 2B and 2C, respectively), compared with concordant subjects. Proportions of activated and naive CD8⁺ T cell subsets did not significantly differ in discordant versus concordant subjects during ART. In addition, memory CD4⁺ and CD8⁺ T cells, which were defined as CD45RA⁺CD62L⁻ and CD45RA⁺CD62L⁻, also did not differ between groups (data not shown).

When immune cell subsets were expressed in absolute cell counts, discordant subjects had significantly higher pretherapy numbers of activated CD4⁺ T cells (\( P = .02 \); figure 2D) and CD8⁺ T cells (530 vs. 375 cells/mm³; \( P = .007 \)) than did concordant subjects. Memory (CD45RA⁺CD62L⁻) CD4⁺ T cell counts (figure 2E; \( P = .02 \)) and memory (CD45RA⁺CD62L⁻) CD8⁺ T cell counts (median, 233 vs. 167 cells/mm³; \( P = .02 \)) were also higher at baseline in discordant versus concordant subjects. In contrast, naive CD4⁺ and CD8⁺ T cell counts at baseline were not different between groups. However, during viral suppression, discordant subjects recovered significantly more naive CD4⁺ (figure 2F) and CD8⁺ T cells at weeks 12 (228 vs. 188 CD8⁺ T cells/mm³; \( P = .02 \)), 24 (247 vs. 182 CD8⁺ T cells/mm³; \( P = .03 \)), and 48 (299 vs. 164 CD8⁺ T cells/mm³; \( P < .01 \)) than did discordant subjects. These data suggest that differences in T cell recovery between subjects developed shortly after ART was initiated (week 12) and that recovery was driven primarily by naive T cells.

**Cellular HIV-1 DNA levels and immunologic discordance during viral suppression.** Increased HIV-1 DNA levels have been reported as being predictive of disease progression and associated with lower CD4⁺ T cell count increases during ART [16, 34–36]. We sought to determine a possible association between cell-associated HIV-1 and immune discordance during viral suppression. HIV-1 DNA was quantified in PBMCs in a randomly selected cohort of 30 discordant and 30 concordant subjects before and after 48 weeks of ART (figure 3).

Discordant and concordant groups had similar HIV-1 DNA levels at baseline (median, 1.67 vs. 1.90 log₁₀ HIV-1 DNA copies/µg of genomic DNA, respectively; \( P = .24 \)) and at week 48 (median, 1.18 vs. 1.54 log₁₀ HIV-1 DNA copies/µg of genomic DNA, respectively; \( P = .39 \)). There were no significant associations between immune discordance and the HIV-1 DNA level at either baseline (\( P = .32 \)) or week 48 (\( P = .42 \)). Furthermore, the HIV-1 DNA level did not correlate with the percentage of naive or memory CD4⁺ T cells recovered during viral suppression (data not shown). Considering that HIV preferentially infects HIV-specific CD4⁺ T cells [2], individuals with different CD4⁺ T cell counts potentially may have different HIV-1 DNA levels when HIV-1 DNA is measured in PBMCs. We therefore adjusted HIV-1 DNA levels on the basis of CD4⁺ T cell counts. In a similar analysis, there were no significant differences in HIV-1 DNA log₁₀ copies/µg of CD4⁺ T cell DNA between concordant and discordant subjects either at baseline or after 48 weeks of suppressive ART (data not shown).
Figure 2. Longitudinal immunophenotypic characteristics during 48 weeks of viral suppression. Wilcoxon rank sum tests were used for bivariate comparisons between immunologic discordant (demonstrating an increase of <100 CD4+ T cells/mm³) (black bars) and concordant (demonstrating an increase of ≥100 CD4+ T cells/mm³) (white bars) subjects in this analysis. A, Median percentages of activated CD4+ T cells (CD4+CD38+HLA-DR+). B, Median percentages of naive CD4+ T cells (CD4+CD45RA+CD62L+). C, Median percentages of memory CD4+ T cells (CD4+CD45RA−CD62L−). D, Median absolute cell counts of activated CD4+ T cells. E, Median absolute cell counts of memory CD4+ T cells. F, Median absolute cell counts of naive CD4+ T cells.
DISCUSSION

Suboptimal CD4+ T cell recovery during ART is a common clinical dilemma. Indeed, of the 116 individuals in this prospective cohort who had viral suppression (≤50 copies/mL), 41% were considered to have immunologic discordance. Whether the mechanisms that underlie this phenomenon are merely a consequence of greater immune damage before initiation of ART or whether they represent differential potentials for immune reconstitution is unclear. Our data support the hypothesis that, in individuals with moderately advanced HIV-1 infection, differential regenerative capacities and previous T cell depletion determine the extent of CD4+ T cell recovery during ART. Furthermore, HIV-1 DNA levels in PBMCs were not associated with immune recovery.

Giorgi et al. [27–29] found that increasing immune activation was associated with disease progression in individuals with untreated HIV-1 infection. Similarly, our data showed higher CD4+ and CD8+ T cell activation at baseline to be predictive of lower CD4+ T cell increases during subsequent viral suppression. At the time of viral control, CD8+ T cell activation decreased to similar levels in both groups, but the percentage of activated CD4+ T cells remained higher in individuals with limited immune restoration. This finding is consistent with previous data showing that lower CD4+ T cell counts in subjects were associated with elevated T cell activation during viral suppression to ≤50 copies/mL [12]. Although an intuitive assumption may be to associate reduced CD4+ T cell recovery with continued activation-induced apoptosis during ART, it is important to emphasize that these differences in CD4+ T cell activation were subtle. Furthermore, consistent with a recent report [37], CD4+ T cell recovery was not influenced by persistent activation in the CD8+ T cell compartment. Although elevated immune activation has been established as a marker of disease progression in untreated patients [27, 28], its role during viral suppression may be less significant.

Initial CD4+ T cell recovery represents a redistribution of sequestered T cells into peripheral circulation, which is mediated by a reduction in immune activation [7, 21]. During first-phase recovery, discordant subjects gained 5 times more naive CD4+ T cells than did discordant subjects. Considering that both groups had similar reductions in immune activation, the extent of T lymphocyte recirculation was likely comparable between groups. Therefore, these differences in first-phase recovery may reflect greater pre-ART immune damage in discordant individuals.

Moreover, naive T cells are disproportionately affected as both aging and HIV-1 infection accelerate depletion [30, 38]. Consistent with published data [14, 20, 22], the findings from our study showed a negative association with age and CD4+ T cell recovery. However, we also observed an independent effect of higher baseline proportions of naive CD4+ T cells and immunologic concordance during ART. Overall, discordant subjects gained a median of 111 naive CD4+ T cells/mm³, compared with an increase of 22 naive CD4+ cells/mm³ in discordant subjects, whereas CD4+ T cell recovery in discordant subjects was driven primarily by memory cells. Interestingly, only the CD45RA+CD62L− subset was associated with suboptimal recovery, because subjects with suboptimal recovery generally had higher proportions of this subset of memory cells than did discordant subjects. Although we cannot differentiate between thymic output and extrathymic expansion, discordant subjects retained greater immune regenerative capacity, as was evidenced by increasing numbers of naive CD4+ T cells through week 48 of ART.

Alternatively, poor immune recovery during ART may be a consequence of continued CD4+ T cell cytolysis resulting from residual viral replication. Total HIV-1 DNA in PBMCs has been associated with low-grade viral replication during potent ART [26, 39] and has been used as a measure of the viral reservoir in several reports [16, 34, 36]. In our study, discordant and discordant subjects had similar amounts of CD4+ T cell recovery. Adjusting HIV-1 DNA levels for differences in CD4+ T cell counts among individuals also failed to demonstrate significant differences between groups. However, it is important to note that our assay detects both integrated and unintegrated forms of cellular HIV-1 DNA and cannot distinguish replication-competent virus from noninfectious particles. Therefore, total HIV-1 DNA, as a measure of the viral
reservoir, is based on the assumption that the proportion of CD4+ T cells with integrated genomes capable of viral replication is similar among untreated individuals. If, however, differences exist, then a more precise, albeit less clinically practical, approach would be to first sort CD4+ T cells and then perform terminal dilution cocultures to quantify the “infectious” viral reservoir. Although several methods can be used to measure the viral reservoir, the usefulness of surrogate markers of residual viremia during suppressive ART is uncertain. In a previous report, intensification of therapy for subjects with plasma HIV-1 RNA levels of ≤50 copies/mL did not result in increases in CD4+ T cells [26].

This retrospective analysis had several limitations—most notably, the arbitrary definition of immunologic concordance. Although a biologically significant definition of immune discordance is unknown, several studies have used similar CD4+ T cell count cutoffs [14, 15, 18, 40]. Furthermore, when multivariate models used CD4+ T cell recovery as a continuous variable, associations were no longer significant. This may represent the nonlinear rate of CD4+ T cell recovery during ART, which is better analyzed using logistic regression. In addition, we found stronger associations by use of immune cell subset proportions rather than absolute cell counts. Considering that only a small percentage of all T lymphocytes traffic to the peripheral circulation, as well as the high prevalence of cytopenias in HIV-infected individuals [41], the proportion of T cell subsets may be a better comparator between subjects than the absolute number of cells.

To our knowledge, the present investigation is the largest single-cohort study of viral and immunologic determinants of discordance in antiretroviral-naive subjects starting ART. Because subjects were selected from the same clinical trial, they had similar CD4+ T cell counts, plasma viral levels, and clinical profiles at baseline. Samples were prospectively collected at multiple time points; therefore, we were able to evaluate early and late immunophenotypic and viral responses to suppressive ART. This ability to follow responses over time allows for the determination of causal relationships and provides better insight into the mechanism of immunologic regeneration during ART.

In conclusion, the present study suggests that CD4+ T cell repopulation during viral suppression is a process determined by distinct mechanisms reflecting both the depth of primary immune damage before therapy and the capacity of T cell regenerative mechanisms during ART. Current guidelines recommend initiating ART on the basis of a total CD4+ T cell count of <350 cells/mm³ [17]. Potentially, markers of T cell maturation and activation have an additional clinical role, because they may identify individuals who need to start ART earlier at higher pretherapy CD4+ T cell counts, before they lose the ability to have robust immune recovery. Furthermore, individuals experiencing immune discordance during receipt of suppressive ART may be better treated by immune-based therapies than by intensification of ART.

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