CD4+ T Cell Recovery beyond the First Year of Complete Suppression of Viral Replication during Highly Active Antiretroviral Therapy Is Not Influenced by CD8+ T Cell Activation

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CD38 expression on CD8+ T cells was longitudinally assessed in 31 human immunodeficiency virus (HIV)–infected persons with undetectable plasma viremia who had undergone highly active antiretroviral therapy (HAART) for 12 months and were followed for a mean of 30 months thereafter. Overall, CD4+ T cell counts increased during follow-up, whereas CD38 expression remained stable. However, a subset of patients showed declines in CD38 expression, and, conversely, another subset showed increases in CD38 expression. No association could be found between long-term gains in CD4+ T cells and evolution of CD38 expression. Thus, activation of CD8+ T cells does not seem to be associated with the extent of CD4+ T cell recovery beyond the first year of successful HAART.

The activation of the immune system is a major determinant of depletion of CD4+ T cells during the natural course of HIV infection. Several surface markers are up-regulated on T cells during the process of cell activation, CD38 being one of the most important [1]. Several studies conducted during the last decade have demonstrated the prognostic value of CD38 expression on CD8+ T cells as a surrogate marker of HIV disease progression [2]. The proportion of CD8+CD38+ T cells have predicted the rate of depletion of CD4+ T cells [3], and, in a recent study, these cells—rather than the level of plasma viremia—were highly predictive of CD4+ T cell loss [4].

In drug-naive persons who initiate highly active antiretroviral therapy (HAART) and achieve complete viral suppression, T cell activation decreases in parallel with plasma viremia [5], although the level of CD38 expression on CD8+ T cells does not reach normal values in most cases [6]. Little is known about the relationship between T cell activation and the magnitude of CD4+ T cell restoration in patients undergoing HAART. We recently demonstrated that, during the first 12 months of successful HAART, T cell activation influences the extent of gains in CD4+ T cells [7]. A cross-sectional study has suggested that this can also be the case in patients experiencing prolonged viral suppression while undergoing long-term HAART [8]. To confirm this, we longitudinally examined the evolution of CD38 expression on CD8+ T cells in a group of HIV-infected patients who initiated HAART and maintained undetectable plasma viremia for a long period of follow-up.

Patients, materials, and methods. Thirty-six drug-naive HIV-1–infected patients who initiated HAART and reached an undetectable level of plasma viremia (<50 RNA copies/mL) were included in the study. Follow-up started 12 months after HAART was begun, at which time all patients had undetectable viral loads. From that time, they were followed for a mean of 30 months. Of the 36 patients, 5 showed a detectable level of plasma viremia at distinct time points during follow-up and were excluded from the analysis. All laboratory assays were done at each visit for each patient.

CD4+ T cells were measured using the Coulter TetraOne reagent and an EPICS XL flow cytometer (Coulter Instruments), in accordance with the manufacturer's instructions. CD38 expression on CD8+ T cells was measured using a quantitative flow cytometry assay (Cellquant CD38/CD8-phycocerythrin; Biocytex), as described elsewhere [9]. Two 50-μL aliquots of fresh whole blood were incubated with anti-CD38 and a negative isotypic control, respectively. A 50-μL aliquot of calibrated bead suspension was incubated with the negative isotypic control. All samples were then incubated with a polyclonal anti-mouse IgG conjugated to fluorescein isothiocyanate. Specimens were then incubated with a neutralization solution, and, in the final step, phycoerythrin-conjugated anti-CD8 was used for counterstaining of all samples, except the calibrated beads.
Figure 1. Temporal evolution of CD4+ T cell counts (A) and CD38 expression on CD8+ T cells (B) in 31 HIV-infected patients undergoing highly active antiretroviral therapy (HAART) who maintained an undetectable level of plasma viremia during the entire follow-up period. The same parameters were analyzed in 2 subgroups of patients: those in whom CD38 expression decreased during the study period (C and D), and those in whom it increased (E and F). In all plots, the horizontal axis represents the time undergoing HAART, in months.

Red cells were lysed using the Coulter Immunoprep lysing solution. A minimum of 2500 CD8+ T cells was examined per sample, using an EPICS XL flow cytometer (Coulter Instruments). HIV RNA was quantified in plasma using the third-generation branched DNA assay (Quantiplex; version 3.0; Bayer), which has a lower detection limit of 50 RNA copies/mL.

The normality of distribution for each variable was tested using a Kolmogorov-Smirnov test. To improve normality, the data were log transformed. The slopes for the different parameters analyzed (activation of CD8+ T cells and CD4+ T cell counts) were calculated using a linear regression analysis. Evolution of CD4+ T cell counts in the whole population was described using a Lowess procedure that uses local regressions to draw a representative smooth curve [10]. Differences between means were assessed using Student’s t test. The association between variables was explored using Pearson’s correlation coefficient, and a multivariate linear regression analysis was used to explore which variables were more strongly associated with the slopes for CD38 expression and CD4+ T cells. All statistical analyses were done using SPSS (version 9.0; SPSS). Data are presented as means ± SD.

Results. All patients were drug-naive when they started HAART. The mean CD4+ T cell count before initiation of HAART was 270 ± 198 cells/µL, and the mean plasma HIV RNA load was 4.6 ± 0.8 log copies/mL. One-quarter of patients were coinfected with hepatitis C virus (HCV). After the first year of HAART, patients were followed for a mean of 30 ± 7 months, with a mean of 6.7 ± 2.1 time points per patient. Eighty percent of subjects were treated with a nonnucleoside reverse-transcriptase inhibitor–based regimen.

At the beginning of follow-up (12 months after initiation of HAART), the mean CD4+ T cell count was 416 ± 220 cells/µL, and the mean level of CD38 expression on CD8+ T cells was 2106 ± 1396 molecules/cell. CD4+ T cell counts significantly increased for most patients, with a mean slope of 7.7 ± 7.9 cells/µL/month (figure 1A). CD4+ T cell counts were significantly
higher at the end of follow-up (from 416 ± 220 to 646 ± 224 cells/μL; P < .001; mean change, 220 ± 185 cells/μL). In contrast, the expression of CD38 on CD8+ T cells did not change significantly during the same period (from 2106 ± 1396 to 2060 ± 1494 molecules/cell) (figure 1B).

A linear regression analysis was performed for each individual patient, using CD38 expression as the dependent variable and time as the explanatory variable. The CD38 expression slope was negative for 55% of the patients and was positive for the others. A linear regression analysis that considered these 2 groups separately revealed statistically significant CD38 expression slopes over time (for patients with negative slopes, −36 ± 17 molecules/cell/month [P < .05]; for patients with positive slopes, 44 ± 14 molecules/cell/month [P < .005]) (figure 1C and 1E). At the end of follow-up, patients with positive CD38 expression slopes had a significantly higher level of CD38 expression than did those with negative slopes (2683 ± 1453 vs. 1547 ± 1360 molecules/cell; P < .05).

Both groups of patients were comparable with respect to several baseline characteristics, including duration of follow-up, pre-HAART CD4+ T cell count, pre-HAART plasma viremia, CD4+ T cell count at the initiation of follow-up, and level of CD38 expression. However, HCV coinfection tended to be more frequent in the group with positive CD38 expression slopes than in the group with negative slopes (38% vs. 8%), although the difference was not statistically significant. In a multivariate regression model that included all of the variables mentioned above, baseline CD38 level was the only predictor of the slope for CD38 expression (table 1).

In both groups of patients, CD4+ T cell counts significantly increased during follow-up (from 389 ± 219 to 619 ± 208 cells/μL and from 450 ± 215 to 677 ± 246 cells/μL for those with negative and positive CD38 expression slopes, respectively) (figure 1D and 1F); however, the mean increases were very similar in both groups (213 ± 124 and 229 ± 248 cells/μL, respectively). In a univariate correlation model, the CD4+ T cell count slope was not associated with the CD38 expression slope. The only parameters significantly correlated with the CD4+ T cell count slope were CD4+ T cell count at the beginning of follow-up and CD4+ T cell gain during the first 12 months of HAART (Pearson’s $r = −0.36$ and $r = −0.44$, respectively; $P < .05$) (table 1). In a multivariate analysis that included pre-HAART CD4+ T cell count, pre-HAART plasma viremia, CD4+ T cell count at the beginning of follow-up, CD4+ T cell gain during the first 12 months of HAART, CD38 expression slope, and HCV coinfection, the only variable that was significantly associated with the CD4+ T cell count slope was CD4+ T cell gain during the first 12 months of HAART (table 1).

**Discussion.** We have longitudinally analyzed the potential association between evolution of immune activation and extent of gains in CD4+ T cells in HIV-infected patients with complete viral suppression who were followed for long periods of time. The role played by T cell activation in the evolution of CD4+ T cell counts in patients undergoing HAART has only recently been explored. Treatment failure has been associated with increases in T cell activation and further declines in CD4+ T cells [11]. Even low-level but persistent plasma viremia in patients undergoing HAART may induce T cell activation and limit CD4+ T cell recovery [12]. In contrast, in patients achieving good control of viral replication with HAART, T cell activation declines and continues to decrease even after prolonged periods of complete viral suppression [9]. In these patients, T cell activation may reflect residual viral replication and therefore may influence the extent of CD4+ T cell recovery. We recently demonstrated that gains in CD4+ T cells in these patients are influenced by the amount of CD38 expressed on CD8+ T cells [7]. It is unknown whether subsequent gains in CD4+ T cells in patients undergoing long-term successful HAART may likewise be modulated by CD8+ T cell activation.

We did not find any association between the evolution of CD38 expression and CD4+ T cell counts in patients maintaining complete viral suppression beyond the first year of successful HAART. The only factor associated with CD4+ T cell gains in the long term was the extent of restoration of CD4+ T cells obtained during the first 12 months of therapy. The negative sign of this association means that the higher the increase in CD4+ T cells during the first year, the lower it was afterward. Because CD4+ T cell gains tend to plateau after the first 2 years of therapy [13], this plateau might be reached earlier in patients with higher CD4+ T cell slopes during the first year of HAART.

The lack of association between T cell activation and changes in CD4+ T cell counts in the long term contrasts with the results of a recent study by Hunt et al. [8]. However, patients in that study were followed, on average, for 27 months after initiating HAART, whereas our follow-up extended for 42 months total. Because most of the CD4+ T cell restoration occurs during the first 2 years of HAART, it is likely that, beyond that time, any influence of T cell activation on CD4+ T cell recovery might be weaker or vanish as long as plasma viremia remains undetectable, as was the case in our patients. Most importantly, plasma viremia did not remain undetectable at all time points in Hunt et al.’s patients [8], with 20% of their samples showing detectable HIV RNA. Low-level viremia and viral blips can induce an up-regulation of T cell activation [9], and, most likely, Hunt et al.’s patients had higher and more fluctuating levels of T cell activation than did our population.

Several factors may account for the lack of association between T cell activation and long-term evolution of CD4+ T cell counts. First, although patients could be separated into 2 groups on the basis of evolution of CD38 expression, the fluctuations in this marker were very small in most patients; therefore,
Table 1. Factors associated with the CD38 expression slope and the CD4⁺ T cell count slope in patients with long-term HIV suppression undergoing highly active antiretroviral therapy (HAART).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Change in CD38 expression slope</th>
<th>Change in CD4⁺ T cell count slope</th>
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<tr>
<td></td>
<td>Univariate analysis, mean (95% CI), molecules/cell/month &amp; P</td>
<td>Multivariate analysis, mean (95% CI), molecules/cell/month &amp; P</td>
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<tr>
<td></td>
<td>Univariate analysis, mean (95% CI), cells/μL/month &amp; P</td>
<td>Multivariate analysis, mean (95% CI), cells/μL/month &amp; P</td>
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<td>Each increase of 100 CD4⁺ T cells/μL in pre-HAART CD4⁺ T cell count</td>
<td>6.5 (−1.4 to 17) NS</td>
<td>7.9 (−4.9 to 20.7) NS</td>
</tr>
<tr>
<td>Each increase of 1 log copies/mL in pre-HAART plasma HIV RNA load</td>
<td>−13.4 (−40.2 to 13.3) NS</td>
<td>−5.7 (−31.3 to 20) NS</td>
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<tr>
<td>Each increase of 100 cells/μL in CD4⁺ T cell count at the beginning</td>
<td>4.9 (−3.7 to 13.5) NS</td>
<td>−5 (−18.7 to 8.6) NS</td>
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<tr>
<td>of follow-up</td>
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<tr>
<td>Each increase of 100 cells/μL in the gain in CD4⁺ T cells during the</td>
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<td>first 12 months of HAART</td>
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<tr>
<td>Each increase of 100 molecules/cell in CD38 expression at the beginning</td>
<td>−1.9 (−3 to −0.7) .003</td>
<td>−2.8 (−4.4 to −1.73) .001</td>
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<tr>
<td>of follow-up</td>
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<td>Each increase of 10 molecules/cell/month in the CD38 expression slope</td>
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<tr>
<td>Presence of hepatitis C virus coinfection</td>
<td>29.8 (−21.9 to 81.4) NS</td>
<td>29.7 (−21.1 to 80.5) NS</td>
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<tr>
<td></td>
<td>−0.9 (−9.2 to 7.3) NS</td>
<td>−1.9 (−10 to 6.1) NS</td>
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**NOTE.** CI, confidence interval; NS, not significant (P > .05).
assuming the existence of any association, a larger sample size would be needed to reveal it. However, even if a real effect exists, it must be very small, and other factors are likely more important in determining the evolution of CD4+ T cell counts in the long term. Thus, even assuming that our study could not detect a subtle effect because of sample size, any influence of CD8+ T cell activation on CD4+ T cell recovery in the long term must be considered of minimal clinical impact. Second, the impact of CD8+ T cell activation on CD4+ T cell recovery may vanish after long periods of successful HAART, a hypothesis supported by a recent study that showed that CD4+ T cell recovery in patients with undetectable viremia is not influenced by the extent of viral suppression <50 copies/mL but rather by the occurrence of viral blips [14]. Last, even assuming that patients with higher CD38 expression on CD8+ T cells might experience lower CD4+ T cell gains, other factors not considered in our study might compensate for it, such as a higher thymic output [15].

In conclusion, our results suggest that, in patients undergoing successful HAART, CD4+ T cell counts evolve independently of CD8+ T cell activation in the long term. Therefore, other factors must be sought to explain the interindividual variability in CD4+ T cell recovery in the setting of prolonged, successful HAART.

References


14. Marcelin A, Martínez V, Morini J. Very low levels of plasma HIV-1 viremia in subjects with sustained suppression of viremia <50 copies/ml can influence the recovery of CD4 cell counts after initiating HAART promoting the occurrence of transient viremic episodes. Antiviral Ther 2004; 9(Suppl):72.