

# T Cell Activation Is Associated with Lower CD4<sup>+</sup> T Cell Gains in Human Immunodeficiency Virus–Infected Patients with Sustained Viral Suppression during Antiretroviral Therapy

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Although T cell activation is associated with disease progression in untreated human immunodeficiency virus type 1 (HIV-1) infection, its significance in antiretroviral-treated patients is unknown. Activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) T cell counts were measured in 99 HIV-infected adults who had maintained a plasma HIV RNA level  $\leq 1000$  copies/mL for a median of 21 months while receiving antiretroviral therapy. Patients with sustained viral suppression had lower levels of T cell activation than untreated patients but higher levels than HIV-uninfected control subjects. Persistent T cell activation was associated with decreased CD4<sup>+</sup> T cell gains during therapy. For every 5% increase in the proportion of activated CD8<sup>+</sup> T cells, 35 fewer CD4<sup>+</sup> T cells/mm<sup>3</sup> were gained. Increased T cell activation was associated with shorter duration of viral suppression, hepatitis C virus coinfection, frequent low-level viremia, and lower nadir CD4<sup>+</sup> T cell counts. Interventions that directly target T cell activation or the determinants of activation may prove to be useful adjuvants to antiretroviral therapy.

Despite clear improvements in morbidity and mortality that are associated with the use of highly active antiretroviral therapy (HAART) [1, 2], human immunodeficiency virus type 1 (HIV-1)–infected patients often do not have normal CD4<sup>+</sup> T cell counts, even after several years of sustained suppression of plasma HIV RNA levels [3, 4]. Furthermore, although most patients experience CD4<sup>+</sup> T cell gains while receiving HAART, some experience a plateau in CD4<sup>+</sup> T cell gains shortly after initiation of therapy [5]. Differences in thymic function likely explain some of this variability in im-

munologic responses [3, 6–9], but much is still unexplained.

Because T cell activation is strongly associated with clinical progression to AIDS and death in untreated HIV-infected patients [10–12], we postulated that ongoing T cell activation might also limit the immunologic responses among patients with sustained HAART-mediated viral suppression. In patients with untreated

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Written informed consent was obtained from all participants, and the human experimentation guidelines of the US Department of Health and Human Services and of the University of California, San Francisco, were followed. The institutional review board of the University of California, San Francisco, approved the study.

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HIV infection, levels of T cell activation are generally high [13], which results in rapid proliferation and apoptosis [14–17], enhanced CD4<sup>+</sup> T cell susceptibility to infection [18–22], and enhanced HIV replication in infected cells [23–25]. T cell activation is thought by many to be the proximate cause of CD4<sup>+</sup> T cell depletion in untreated patients [26–30]. Although less is known about T cell activation among patients treated with HAART, abnormal levels of T cell activation may persist even after 3 years of sustained viral suppression [4].

To address the question of whether persistent T cell activation affects immune reconstitution in HIV-infected patients with viral suppression during HAART, we identified patients who had maintained viral suppression for  $\geq 3$  months, measured activated T cell subsets by flow cytometry, and assessed the relationship between T cell activation and the overall CD4<sup>+</sup> T cell changes experienced during HAART. We also evaluated factors associated with T cell activation.

## SUBJECTS AND METHODS

**Participants.** Participants were selected from the Study of the Consequences of the Protease Inhibitor Era (SCOPE), a clinic-based cohort of 450 chronically HIV-infected patients who receive primary care in HIV/AIDS specialty clinics at either San Francisco General Hospital or the San Francisco Veterans Affairs Medical Center. The cohort was assembled to represent distinct groups of HIV-infected persons: 25% of participants were untreated at enrollment; 50% were receiving HAART but were in a state of virologic failure at enrollment, with plasma HIV RNA levels consistently  $>1000$  copies/mL for at least 24 weeks; and 25% were receiving HAART at enrollment and had been experiencing virologic suppression with plasma HIV RNA levels consistently  $<50$  copies/mL for at least 24 weeks, with allowance for occasional transient detectable viremia  $\leq 1000$  copies/mL. Participants are seen at 4-month intervals, at which times a detailed questionnaire that collects information about antiretroviral use and risk behavior is administered and blood and saliva specimens are obtained. The first 175 consecutive SCOPE participants had immunophenotyping of T cells performed at the second study visit. Participants were included in the present analysis if they were receiving HAART, had a plasma HIV RNA level  $\leq 1000$  copies/mL on the day of immunophenotyping and for at least 3 months before immunophenotyping, and had a documented CD4<sup>+</sup> T cell count determination within 6 months before the initiation of HAART. We included participants with plasma HIV RNA levels that were detectable (50–1000 copies/mL), because intermittent episodes of low-level viremia are commonly observed in patients who have durable viral suppression [31], and such events generally do not result in treatment modification [32, 33]; in addition, we were interested in examining the relationship between low-

level viremia and T cell activation. Patients were not excluded for intercurrent illnesses or immunizations. HAART was defined as a regimen of  $\geq 3$  antiretroviral drugs that included a protease inhibitor, a nonnucleoside reverse-transcriptase inhibitor, or abacavir. Saquinavir hard gel capsule-based regimens were considered to be HAART only if they were combined with zidovudine. Thirteen untreated HIV-infected participants and 6 healthy HIV-uninfected volunteers were also included, as comparison groups.

**Measurements.** Information about demographic characteristics and antiretroviral medication use at the time of and after SCOPE enrollment was collected by structured interview; medication use before cohort enrollment was determined by medical chart abstraction. Plasma HIV RNA levels were determined by the bDNA amplification technique (Quantiplex HIV RNA; Chiron), either during the course of clinical care, before entry into the cohort (versions 2.0 and 3.0), or at every 4-month study-related visit (all version 3.0). Hepatitis C virus serostatus was determined by Abbott Hepatitis C Virus Enzyme Immunoassay version 2.0 (Abbott Laboratories). CD4<sup>+</sup> T cell counts were measured by the clinical laboratories associated with each medical center. Pretherapy CD4<sup>+</sup> T cell counts and plasma HIV RNA levels were defined as the last available measurement before initiation of HAART. The nadir CD4<sup>+</sup> T cell count was the lowest confirmed value before initiation of HAART.

Immunophenotyping was performed using freshly collected, EDTA-anticoagulated whole blood and analyzed by 4-color flow cytometry on a Beckman Coulter Epics XL flow cytometer. Blood was stained and lysed on a Beckman Coulter Prep Plus and a Beckman Coulter TQ Prep. Activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) T cells were identified using fluorescein isothiocyanate (FITC)-conjugated anti-HLA-DR, phycoerythrin (PE)-conjugated anti-CD38, PE-cyanin red 5.1 (PC5)-conjugated anti-CD3, and PE-Texas red (ECD)-conjugated anti-CD4 or CD8. CD3 was included in the definition of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells to exclude monocytes and NK cells. The activation markers CD38 and HLA-DR were gated from the CD4<sup>+</sup> and CD8<sup>+</sup> cells on a 2-dimensional dot plot in which quadrant gates, set on an isotype control, were used to define the positive and negative populations. Naive (CD45RA<sup>+</sup>CD62L<sup>+</sup>) T cell populations were identified using FITC-conjugated anti-CD45RA, PE-conjugated anti-CD62L, PC5-conjugated anti-CD3, and ECD-conjugated anti-CD4 or CD8. Activated and naive phenotypes were reported as the percentage of CD4<sup>+</sup> or CD8<sup>+</sup> lymphocytes expressing the relevant surface markers.

**Statistical analysis.** Differences in the degree of CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation were compared with a Kruskal-Wallis test among the HIV-infected participants receiving suppressive HAART, the untreated participants, and the healthy HIV-un-

infected volunteers. Wilcoxon rank sum tests were used for pairwise comparisons.

The date of viral suppression was defined as the midpoint between the date of the last plasma HIV RNA level >1000 copies/mL measured during HAART (or the date of HAART initiation, if there were no plasma HIV RNA levels >1000 copies/mL during HAART) and the date on which the first subsequent plasma HIV RNA level  $\leq$ 1000 copies/mL was measured. If a participant achieved viral suppression and then had a plasma HIV RNA level >1000 copies/mL, the date of viral suppression was determined after the last measurement of a plasma HIV RNA level >1000 copies/mL. The duration of viral suppression was defined as the time interval between the date on which viral suppression was achieved and the date of flow cytometry. The overall frequency of low-level viremia during virologically suppressive HAART was defined as the percentage of all plasma HIV RNA measurements made after the date of viral suppression up to and including the date of immunophenotyping that were detectable (50–1000 copies/mL). The frequency of low-level viremia in the year before immunophenotyping was defined as the percentage of all plasma HIV RNA measurements in the year before immunophenotyping and after the date of viral suppression that were detectable (50–1000 copies/mL).

Because the early gains in peripheral CD4<sup>+</sup> T cell counts that occur after initiation of HAART are largely the result of redistribution from lymphoid tissue [34–36], we analyzed separately the factors associated with early and late CD4<sup>+</sup> T cell count changes during HAART. For each participant, the early change in CD4<sup>+</sup> T cell count was calculated by subtracting the pretherapy CD4<sup>+</sup> T cell count from the CD4<sup>+</sup> T cell count at month 3 of HAART (median count within a 10-week window). The late change in CD4<sup>+</sup> T cell count was calculated by subtracting the CD4<sup>+</sup> T cell count at month 3 of HAART from the CD4<sup>+</sup> T cell count at the time of immunophenotyping (median count within a 3-month window). For participants in whom viral suppression occurred after month 3 of HAART, the late CD4<sup>+</sup> T cell changes were calculated by subtracting the CD4<sup>+</sup> T cell count at the date of viral suppression (median count within a 3-month window) from the CD4<sup>+</sup> T cell count at the time of immunophenotyping.

Factors associated with early and late HAART-mediated CD4<sup>+</sup> T cell count changes were analyzed using linear regression. Percentages of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells, age, sex, pretherapy CD4<sup>+</sup> T cell count, log<sub>10</sub> pretherapy plasma HIV RNA level, and hepatitis C virus serostatus were all considered to be potentially associated with early CD4<sup>+</sup> T cell count changes. In addition to each of these factors, the percentage of naive CD4<sup>+</sup> T cells, the time maintaining viral suppression, and the frequency of low-level viremia were considered to be potentially associated with late CD4<sup>+</sup> T cell count changes. All

factors considered were included in a single multivariable model. In these analyses and all subsequent regression analyses, model residuals were evaluated to assess their conformity with the assumptions of linear regression.

Factors associated with CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation among patients with HAART-mediated viral suppression were also assessed using linear regression. The duration of viral suppression, hepatitis C virus serostatus, the frequency of low-level viremia in the year before immunophenotyping, and the nadir CD4<sup>+</sup> T cell count were all considered to be potentially associated with both CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation. The frequency of low-level viremia in the year before immunophenotyping was categorized in modeling both CD4<sup>+</sup> and CD8<sup>+</sup> activation markers to meet the model assumptions. All factors considered were included in a single multivariable model. All analyses were performed with STATA version 7.0.

## RESULTS

**Baseline characteristics.** A total of 99 HAART-treated participants who had maintained plasma HIV RNA levels  $\leq$ 1000 copies/mL for at least 3 months before immunophenotyping were included in the analysis. The majority were men between the ages of 40 and 50 years who were receiving a protease inhibitor-based HAART regimen (table 1). The median pretherapy CD4<sup>+</sup> T cell count was 210 cells/mm<sup>3</sup> (interquartile range [IQR], 84–362 cells/mm<sup>3</sup>); 28% of participants were coinfecting with hepatitis C virus; and the median duration of viral suppression was 21 months (IQR, 11–36 months). The median frequency of plasma HIV RNA measurements that were detectable (50–1000 copies/mL) was 20% (IQR, 5%–44%).

**Abnormal levels of T cell activation despite HAART-mediated viral suppression.** After a median of 21 months of viral suppression, the HAART-treated patients had a lower median percentage of activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) CD4<sup>+</sup> T cells (4%; IQR, 3%–7%) than untreated HIV-infected participants (8%; IQR, 7%–15%;  $P < .001$ ) but a higher median percentage than HIV-uninfected volunteers (1%; IQR, 1%–2%;  $P < .001$ ) (figure 1A). Similarly, the HAART-treated patients had a lower median percentage of activated CD8<sup>+</sup> T cells (11%; IQR, 7%–18%) than untreated HIV-infected participants (46%; IQR, 31%–51%;  $P < .001$ ) but a higher percentage than HIV-uninfected volunteers (1%; IQR, 1%–3%;  $P < .001$ ) (figure 1B). Participants with HAART-mediated viral suppression continued to have higher levels of both CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation than the HIV-uninfected control subjects, even when the analysis was restricted to the 58 participants who had negative results of serologic testing for hepatitis C virus or the 68 participants who had maintained undetectable plasma HIV RNA levels in the year before immunophenotyping ( $P < .001$  for all comparisons).

**Table 1. Characteristics of 99 human immunodeficiency virus (HIV)-infected patients receiving highly active antiretroviral therapy (HAART) who maintained a plasma HIV RNA level  $\leq 1000$  copies/mL for at least 3 months.**

Characteristic	Value
Age, median years (IQR)	45 (40–50)
Male sex, %	90
Nadir CD4 <sup>+</sup> T cell count, median cells/mm <sup>3</sup> (IQR)	106 (50–213)
Months between nadir CD4 <sup>+</sup> T cell count and HAART initiation, median (IQR)	8 (1–30)
Pretherapy CD4 <sup>+</sup> T cell count, median cells/mm <sup>3</sup> (IQR)	210 (84–362)
Pretherapy plasma HIV RNA level, <sup>a</sup> median log <sub>10</sub> copies/mL (IQR)	4.4 (3.7–5.0)
Percentage of patients who were hepatitis C antibody positive <sup>b</sup>	28
Percentage of patients receiving indicated HAART regimen	
Protease inhibitor based	44
NNRTI based	19
Both protease inhibitor and NNRTI based	37
Frequency of low-level viremia, <sup>c</sup> median % (IQR)	20 (5–44)
Interval between initiation of HAART and immunophenotyping, median months (IQR)	27 (17–39)
Duration of viral suppression, <sup>d</sup> median months (IQR)	21 (11–36)

**NOTE.** IQR, interquartile range; NNRTI, nonnucleoside reverse-transcriptase inhibitor.

<sup>a</sup> Data were available for only 97 patients.

<sup>b</sup> Data were available for only 79 patients.

<sup>c</sup> Percentage of all plasma HIV RNA measurements after the date of viral suppression that were detectable (50–1000 copies/mL) for each participant.

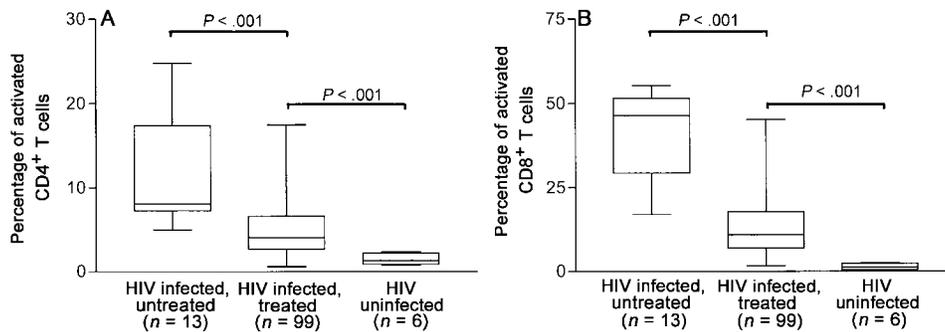
<sup>d</sup> Duration of period before immunophenotyping in which plasma HIV RNA levels were continuously  $\leq 1000$  copies/mL.

**Association between CD4<sup>+</sup> T cell activation and blunted early CD4<sup>+</sup> T cell gains.** The median early CD4<sup>+</sup> T cell count change—between the last measurement before the initiation of HAART and month 3 of HAART—was +53 cells/mm<sup>3</sup> (IQR, +3 to +114 cells/mm<sup>3</sup>). Although the extent of CD4<sup>+</sup> T cell activation was measured, on average, 2 years later, it was found to be associated with these early changes in CD4<sup>+</sup> T cell count. After adjustment for other factors, for every 5% increase in the proportion of activated CD4<sup>+</sup> T cells, 45 fewer CD4<sup>+</sup> T cells/mm<sup>3</sup> were gained between the measurement before initiation of HAART and that at month 3 after initiation of HAART ( $P = .02$ ). Although each 1 log<sub>10</sub> increase in the pretherapy plasma HIV RNA level was associated with an increase of 29 CD4<sup>+</sup> T cells/mm<sup>3</sup> in the gain that occurred during the first 3 months in an unadjusted analysis ( $P = .006$ ), only a trend toward higher early CD4<sup>+</sup> T cell gains was seen in association with higher pretherapy plasma HIV RNA levels (+23 cells/mm<sup>3</sup>

for each increase of 1 log<sub>10</sub> copy/mL) after adjustment for the extent of CD4<sup>+</sup> T cell activation among other factors ( $P = .08$ ). There was no evidence that CD8<sup>+</sup> T cell activation, age, sex, pretherapy CD4<sup>+</sup> T cell count, or hepatitis C virus serostatus was associated with early CD4<sup>+</sup> T cell count changes.

**Association between blunted late CD4<sup>+</sup> T cell gains and CD8<sup>+</sup> T cell activation.** The median late CD4<sup>+</sup> T cell count change—between month 3 after initiation of HAART (or the date of viral suppression, if this occurred later) and the date of immunophenotyping—was +144 cells/mm<sup>3</sup> (IQR, 63–292 cells/mm<sup>3</sup>). In this case, CD8<sup>+</sup> T cell activation, but not CD4<sup>+</sup> T cell activation, was associated with the number of CD4<sup>+</sup> T cells gained. After adjustment for other factors, including the duration of viral suppression, for every 5% increase in the percentage of activated CD8<sup>+</sup> T cells, 35 fewer CD4<sup>+</sup> T cells/mm<sup>3</sup> were gained over the interval ( $P < .001$ ; table 2). The percentage of naive (CD45RA<sup>+</sup>CD62L<sup>+</sup>) CD4<sup>+</sup> T cells and age also were associated with late CD4<sup>+</sup> T cell gains. After adjustment for other factors, for each 5% increase in the percentage of naive CD4<sup>+</sup> T cells, 17 more CD4<sup>+</sup> T cells/mm<sup>3</sup> were gained ( $P = .001$ ). Likewise, for every 10-year increase in age, 50 fewer CD4<sup>+</sup> T cells/mm<sup>3</sup> were gained, after adjustment for other factors ( $P = .02$ ). After adjustment for other factors, 5 more CD4<sup>+</sup> T cells/mm<sup>3</sup> were gained per month of viral suppression ( $P < .001$ ). After adjustment for other factors, there was no evidence that the proportion of activated CD4<sup>+</sup> T cells, sex, pretherapy CD4<sup>+</sup> T cell count, pretherapy plasma HIV RNA level, hepatitis C virus serostatus, and the frequency of low-level viremia were associated with late CD4<sup>+</sup> T cell gains.

**Factors associated with T cell activation during antiretroviral treatment.** We next evaluated factors associated with T cell activation among the 99 patients who had sustained viral suppression while receiving HAART. Levels of CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation were highly correlated ( $\rho = 0.67$ , Spearman's correlation coefficient;  $P < .001$ ). There was also a clear relationship between both CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation and duration of viral suppression. Markers of CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation appear to decrease as duration of viral suppression increases (figure 2). After adjustment for the frequency of low-level viremia in the year before immunophenotyping, among other factors, for every month of viral suppression, there were 0.06% fewer activated CD4<sup>+</sup> T cells ( $P = .03$ ) and 0.1% fewer activated CD8<sup>+</sup> T cells ( $P = .04$ ) (table 3). Participants coinfecting with hepatitis C virus had 2% more activated CD4<sup>+</sup> T cells ( $P = .03$ ) and 5% more activated CD8<sup>+</sup> T cells ( $P = .006$ ) than participants without hepatitis C infection, after adjustment for other factors. Participants with frequent low-level viremia in the year before immunophenotyping (>50% of plasma HIV RNA determinations were detectable (50–1000 copies/mL) had 5% more activated CD8<sup>+</sup> T cells than patients with sustained undetectable plasma HIV RNA levels ( $P = .03$ ),



**Figure 1.** T cell activation in human immunodeficiency virus (HIV)-infected and HIV-uninfected adults. The percentage of activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) CD4<sup>+</sup> T cells (A) and activated CD8<sup>+</sup> T cells (B) was compared between untreated HIV-infected participants, HIV-infected participants who had sustained plasma HIV RNA levels  $\leq$ 1000 copies/mL for a median of 21 months while receiving HAART, and HIV-uninfected volunteers. Each central bar represents the median value, each box represents the 25th through 75th percentile (interquartile) range, and the whiskers include 1.5 times the interquartile range below the 25th-percentile and above the 75th-percentile values.

but similar levels of CD4<sup>+</sup> T cell activation (2% more;  $P = .14$ ) after adjustment for other factors. For each increase of 100 cells/mm<sup>3</sup> in the nadir CD4<sup>+</sup> T cell count, there were 1% fewer activated CD4<sup>+</sup> T cells ( $P = .003$ ) and 1.5% fewer activated CD8<sup>+</sup> T cells ( $P = .05$ ), after adjustment for other factors.

## DISCUSSION

Although most HIV-infected patients experience sustained CD4<sup>+</sup> T cell gains while receiving virologically suppressive HAART, some patients never experience robust CD4<sup>+</sup> T cell gains [5], and others fail to achieve a normal CD4<sup>+</sup> T cell count even after several years of therapy [3]. Because T cell activation is predictive of disease progression in the untreated patients [10, 11, 37], even independent of virus load [12, 38], we hypothesized that abnormal T cell activation might persist in patients experiencing long-term viral suppression and might be associated with the immunologic response to therapy. Among HIV-infected participants with a median of almost 2 years of HAART-mediated viral suppression, we found substantial levels of both CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation, well above the levels observed in HIV-uninfected persons. Furthermore, higher levels of CD4<sup>+</sup> T cell activation were independently associated with lower CD4<sup>+</sup> T cell gains experienced in the first 3 months of therapy, and higher levels of CD8<sup>+</sup> T cell activation were independently associated with lower CD4<sup>+</sup> T cell gains after month 3. Our results provide further support for the hypothesis that T cell activation plays a critical role in HIV pathogenesis.

It has long been proposed that T cell activation may be a mechanism by which HIV infection leads to CD4<sup>+</sup> T cell depletion. Given the small proportion of T cells that are actually infected by HIV [39], it has been inferred that mechanisms other than direct loss of infected CD4<sup>+</sup> T cells must play a role in CD4<sup>+</sup> T cell depletion [30, 40–42]. Indeed, HIV infection

activates uninfected, resting T cells either indirectly, via inflammatory cytokines [43, 44], or directly, via contact with antigen-presenting cells presenting HIV antigens [18, 19, 45, 46] or exposure to gp120 and other viral factors [47]. These activated T cells undergo rapid proliferation [14–16] and are prone to spontaneous apoptosis [17, 41, 42]. Normally, some progeny of T cells activated by an antigenic stimulus revert back to long-lived resting phenotypes after withdrawal of the activating stimulus. However, in the presence of ongoing antigen and inflammatory stimulation by HIV, the resting pool of naive and memory CD4<sup>+</sup> T cells may be continually drained, resulting in an inability to restore the CD4<sup>+</sup> T cells lost as a consequence of HIV infection [30]. This hypothesis is supported by the observation that sooty mangabeys, which fail to develop increased levels of T cell activation and apoptosis in response to simian immunodeficiency virus infection, rarely develop CD4<sup>+</sup> T cell depletion, despite ongoing CD4<sup>+</sup> T cell infection and destruction by simian immunodeficiency virus [48].

Our finding that increased CD8<sup>+</sup> T cell activation is associated with lower CD4<sup>+</sup> T cell gains after 3 months of HAART-mediated viral suppression is consistent with other work relating CD8<sup>+</sup> T cell activation to disease progression in untreated patients [10–12, 17]. Interestingly, these studies have generally concluded that markers of CD8<sup>+</sup> activation, but not CD4<sup>+</sup> activation, predict clinical progression, although there is some controversy on this point [38]. One possible explanation for the predictive superiority of CD8<sup>+</sup> T cell activation markers is related to differential rates of HIV infection observed in activated T cells. Because activated CD4<sup>+</sup> T cells are preferentially infected by HIV [19–22, 49], their half-lives are determined not only by the rate of activation-induced apoptosis but also by the rate of HIV infection and direct virus-mediated cell death [50]. Higher levels of HIV replication might reduce the percentage of activated CD4<sup>+</sup> T cells by accelerating their removal, and therefore CD8<sup>+</sup> T cell activation markers can be expected

**Table 2. Factors associated with late changes in CD4<sup>+</sup> T cell gains in human immunodeficiency virus–infected patients.**

Factor	Unadjusted analysis		Adjusted analysis <sup>a</sup>	
	Mean change (95% CI) in CD4 <sup>+</sup> T cell gains, cells/mm <sup>3</sup>	<i>P</i>	Mean change (95% CI) in CD4 <sup>+</sup> T cell gains, cells/mm <sup>3</sup>	<i>P</i>
Each 5-percentage-point increase in percentage of (CD38 <sup>+</sup> HLA-DR <sup>+</sup> ) CD8 <sup>+</sup> T cells	−45 (−64 to −25)	<.001	−35 (−62 to −20)	<.001
Each 5-percentage-point increase in percentage of (CD45RA <sup>+</sup> CD62L <sup>+</sup> ) CD4 <sup>+</sup> T cells	+15 (4–26)	.01	+17 (7–26)	.001
Each 5-percentage-point increase in percentage of (CD38 <sup>+</sup> HLA-DR <sup>+</sup> ) CD4 <sup>+</sup> T cells	−61 (−108 to −14)	.01	+18 (−33 to 70)	.47
Each 1-month increase in the duration of viral suppression <sup>b</sup>	+6 (4–8)	<.001	+5 (3–7)	<.001
Each 10-year increase in age	−37 (−88 to 13)	.14	−50 (−90 to −7)	.02
Each increase of 100 cells/mm <sup>3</sup> in pretherapy CD4 <sup>+</sup> T cell count	+10 (−10 to 32)	.30	−10 (−30 to 10)	.33
Each increase of 1 log <sub>10</sub> copy/mL in pretherapy plasma HIV RNA level	+15 (−22 to 51)	.43	−3 (−36 to 30)	.85
Sex				
Male	Reference	—	Reference	—
Female	−19 (−137 to 99)	.75	−21 (−135 to 93)	.71
Hepatitis C virus antibody serostatus				
Negative	Reference	—	Reference	—
Positive	−14 (−95 to 67)	.73	+64 (−8 to 135)	.08
Each 10% increase in frequency of low-level viremia <sup>c</sup>	−2 (−3 to −0.5)	.007	+2 (−9 to 14)	.65

**NOTE.** Factors associated with the change in CD4<sup>+</sup> T cell count from month 3 of HAART (or the date on which a plasma HIV RNA level ≤1000 copies/mL was achieved, if this occurred after month 3) to the date of immunophenotyping were assessed using linear regression. Adjusted regression coefficients are reported from a single multivariate model containing all of the listed factors. CI, confidence interval.

<sup>a</sup> *R*<sup>2</sup> = 0.55.

<sup>b</sup> Duration of period before flow cytometry was done in which plasma HIV RNA levels were continuously ≤1000 copies/mL.

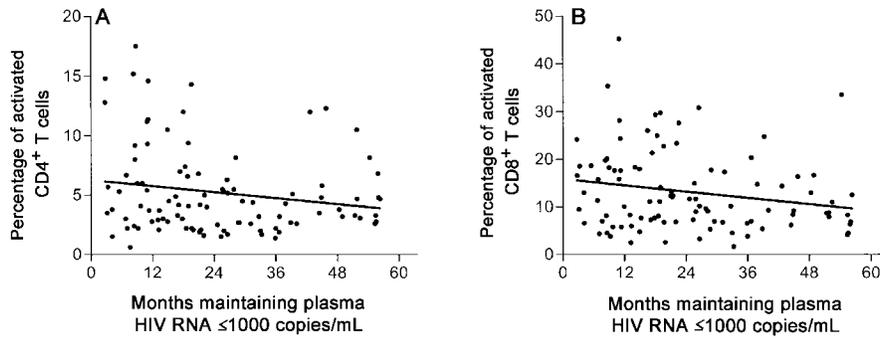
<sup>c</sup> Percentage of all plasma HIV RNA measurements after the date of viral suppression that were detectable (50–1000 copies/mL) for each participant.

to be more closely associated with the ability of HIV to cause generalized T cell activation and, by extension, the rate at which CD4<sup>+</sup> T cells are gained or lost.

It is also interesting that HIV-infected patients maintaining viral suppression in this study had substantially higher levels of T cell activation than did HIV-uninfected control subjects. This difference was observed even when HIV-infected participants with persistent detectable viremia or hepatitis C virus coinfection were excluded. Because the half-life of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells is short [50], the presence of heightened T cell activation after years of suppressive HAART suggests the presence of ongoing antigenic stimulation. This might reflect ongoing low-level HIV replication in lymphoid tissues; the presence of other chronic infections, as a result of continued immunodeficiency (e.g., herpesvirus infections); or persistent immunologic dysregulation that is not reversed by HAART-mediated viral suppression. The former possibility is consistent with a recent study in which treatment intensification among

patients with very low-level viremia (HIV RNA level, 2.5–50 copies/mL) resulted in decreased levels of plasma HIV RNA and decreased levels of T cell activation [51].

Although abnormal levels of T cell activation were observed in our participants, it is notable that the levels appeared to decrease as the duration of viral suppression increased. We did not perform repeated measurements over time and therefore cannot exclude the possibility that patients with high levels of persistent T cell activation were preferentially excluded from our sample because of virologic failure; however, the observed association was not confounded by the extent of low-level viremia and suggests that levels of CD8<sup>+</sup> T cell activation continue to decrease for years after viral suppression is achieved. This might reflect a continued, albeit gradual, decrease in the level of viral replication that remains below the level of detection of standard plasma HIV RNA assays and/or it might reflect the slow decay of the latent reservoir of infected cells. In either case, a slow decrease of CD8<sup>+</sup> T cell activation over time might



**Figure 2.** T cell activation levels during long-term viral suppression. The percentage of activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) CD4<sup>+</sup> T cells (A) and activated CD8<sup>+</sup> T cells (B) for 99 human immunodeficiency virus (HIV)-infected participants receiving highly active antiretroviral therapy are plotted against duration of plasma HIV RNA levels  $\leq 1000$  copies/mL. Linear regression was used to describe the relationships of CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation with time. The predicted values from each model are superimposed as a line on the graph.

be one explanation for the slow, but persistent, CD4<sup>+</sup> T cell gains observed in the majority of patients with long-term HAART-mediated viral suppression [52].

Our finding that persistent CD4<sup>+</sup>, but not CD8<sup>+</sup>, T cell activation during suppressive HAART is associated with decreased CD4<sup>+</sup> T cell gains in the first 3 months of therapy suggests that redistribution of CD4<sup>+</sup> T cells from lymphoid tissue is more closely associated with a decrease in CD4<sup>+</sup> T cell activation than with a decrease in CD8<sup>+</sup> T cell activation. Because most patients with untreated HIV infection have high levels of pre-HAART CD4<sup>+</sup> T cell activation [53], we can assume that

those patients with the lowest levels of CD4<sup>+</sup> T cell activation during suppressive HAART were likely to have had the largest decrease in T cell activation in the first few months of therapy. Because CD4<sup>+</sup> T cell activation is closely associated with cell adhesion molecule [18, 19, 49] and chemokine receptor [20–22] expression, we can speculate that a large reduction in CD4<sup>+</sup> T cell activation is associated with a large redistribution of CD4<sup>+</sup> T cells from lymphoid tissue. The observation of Hejdeman et al. [54], who measured pre- and post-HAART activation levels in a cohort of HIV-infected patients initiating HAART, that patients who experience a reduction in the plasma HIV RNA

**Table 3. Factors associated with changes in activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) T cell counts in 99 human immunodeficiency virus (HIV)-infected patients with sustained plasma HIV RNA levels  $\leq 1000$  copies/mL.**

Factor	CD4 <sup>+</sup> T cells				CD8 <sup>+</sup> T cells			
	Unadjusted analysis		Adjusted analysis <sup>a</sup>		Unadjusted analysis		Adjusted analysis <sup>b</sup>	
	Mean change (95% CI) in activated T cells, %	P	Mean change (95% CI) in activated T cells, %	P	Mean change (95% CI) in activated T cells, %	P	Mean change (95% CI) in activated T cells, %	P
Each 1-month increase in duration of viral suppression <sup>c</sup>	-0.04 (-0.09 to 0.004)	.08	-0.06 (-0.1 to -0.005)	.03	-0.1 (-0.2 to -0.004)	.04	-0.1 (-0.2 to -0.01)	.04
Hepatitis C virus antibody status								
Negative	Reference	—	Reference	—	Reference	—	Reference	—
Positive	+2 (0.3–4)	.02	+2 (0.2–4)	.03	+6 (2–10)	.003	+5 (2–9)	.006
Frequency of low-level viremia in year before immunophenotyping <sup>d</sup>								
None	Reference	—	Reference	—	Reference	—	Reference	—
1%–50%	+0.1 (-2 to 2)	.96	-0.5 (-3 to 2)	.69	-1 (-6 to 4)	.74	-4 (-10 to 2)	.18
>50%	+3 (1–4)	.005	+2 (-0.2 to 4)	.07	+7 (3–11)	.001	+5 (0.5–10)	.03
Each increase of 100 cells/mm <sup>3</sup> in nadir CD4 <sup>+</sup> T cell counts	-0.5 (-1.0 to 0.1)	.11	-1 (-2 to -0.3)	.003	-0.3 (-2 to 1)	.65	-1.5 (-3.0 to -0.03)	.05

**NOTE.** Factors associated with CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation were assessed using linear regression. Adjusted regression coefficients are reported from a single multivariable model including all listed factors.

<sup>a</sup>  $R^2 = 0.25$ .

<sup>b</sup>  $R^2 = 0.27$ .

<sup>c</sup> Time interval between the date of viral suppression and the date of immunophenotyping.

<sup>d</sup> Percentage of all plasma HIV RNA determinations after the date of viral suppression and in the year before immunophenotyping that were detectable. Sixty-eight patients had no low-level viremia; 12 had low-level viremia in 1%–50% of determinations; and 19 had low-level viremia in >50% of determinations.

level of  $<1$  log still experience an early and sustained gain in  $CD4^+$  T cell counts that correlates with a reduction in the level of  $CD4^+$  T cell activation markers despite unchanged levels of  $CD8^+$  T cell activation markers, is consistent with this hypothesis.

Although our primary focus was on T cell activation, our results remain consistent with other research demonstrating an association between thymic function and  $CD4^+$  T cell restoration in patients receiving virologically suppressive HAART [6–9]. In our study, high levels of  $CD8^+$  T cell activation, older age, and decreased levels of naive  $CD4^+$  T cells were all independently associated with blunted late  $CD4^+$  T cell gains. To the extent that age and the percentage of naive  $CD4^+$  T cells are surrogates for thymic function when adjusting for levels of T cell activation, our data support a model in which 2 related but largely independent factors determine the  $CD4^+$  T cell gains experienced by patients with HAART-mediated viral suppression: T cell activation and thymic function.

A potential limitation of our study is the lack of pre-HAART and repeated during-HAART measurements of T cell activation. Because we only measured markers of T cell activation once and measured HAART-mediated  $CD4^+$  T cell changes retrospectively, we cannot prove that high levels of T cell activation predict lower  $CD4^+$  T cell gains. However, existing prospective data on untreated HIV-infected individuals [10–12] show that our interpretation of a causal relationship is plausible. Another potential limitation is the male predominance and high prevalence of protease inhibitor–based regimens in our cohort, which may limit the generalizability of our findings to other patient populations.

Finally, we found several factors to be associated with persistent T cell activation, which suggest potential approaches to optimizing the immunologic responses to virologically suppressive HAART. First, we observed increased levels of  $CD8^+$  T cell activation in participants with lower pre-HAART nadir  $CD4^+$  T cell counts. This observation is consistent with a recent report of decreased responses to vaccination in HAART-treated patients with normal current  $CD4^+$  T cell counts but low  $CD4^+$  T cell nadirs [55] and suggests that initiating therapy earlier in untreated patients might be associated with improved immunologic function. However, because levels of  $CD8^+$  T cell activation may decline even after several years of viral suppression, it is possible that the immunologic perturbations caused by delaying therapy may be reversible. Second, we observed increased  $CD8^+$  T cell activation in participants with frequent low-level viremia. This suggests that further suppression of viral replication resulting from intensification of HAART may provide some marginal immunologic benefits, as has been observed in 2 recent studies [51, 56]. Third, hepatitis C virus coinfection was associated with persistent T cell activation, which confirms the conclusions of work published elsewhere [57]. This obser-

vation suggests that treatment of other chronic inflammatory diseases might improve immunologic responses to HAART. Fourth, because almost all of our participants had abnormal levels of  $CD8^+$  T cell activation, treatment of T cell activation itself with immunomodulatory medications might be beneficial in HAART-treated patients with sustained viral suppression. In a recent trial, patients with early HIV infection who were treated with HAART plus 8 weeks of cyclosporin achieved and maintained much higher  $CD4^+$  T cell counts and levels of HIV-specific immune responses than did patients treated with HAART alone [58]. Although trials of cyclosporin in chronically infected patients with varying degrees of viral suppression have failed to show benefit [59, 60], future trials of immunomodulators may hold promise if they are targeted at chronically infected patients maintaining viral suppression.

In summary, we have shown that abnormal levels of T cell activation exist in most patients experiencing long-term HAART-mediated viral suppression and that the extent of activation is associated with treatment-associated  $CD4^+$  T cell gains. Improving our understanding of how HIV activates the immune system may lead to the development of more-specific adjuvants to HAART that reverse the immunologic perturbations caused by HIV infection.

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