Low-Level Viremia and Proviral DNA Impede Immune Reconstitution in HIV-1–Infected Patients Receiving Highly Active Antiretroviral Therapy

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Background. Immunological and virological consequences of low-level viremia in human immunodeficiency virus (HIV) type 1–infected patients receiving highly active antiretroviral therapy (HAART) remain to be determined.

Methods. For 24 months, 101 HAART-treated, HIV-1–infected patients with HIV RNA levels ≤200 copies/mL were followed prospectively: HIV RNA level and CD4 and CD8 cell counts were investigated every 3 months, and proviral DNA and T cell subsets were investigated every 6 months.

Results. During follow-up, 33 patients had HIV RNA levels ≤20 copies/mL at all visits (uVL patients), whereas 68 patients had HIV RNA levels >20 copies/mL at ≥1 visit (dVL patients) (median increase, 81 copies/mL [interquartile range, 37–480 copies/mL]). dVL patients had higher concentrations of CD8 cells, activated and memory T cells, and proviral DNA, compared with uVL patients (P <.05). A higher HIV RNA level was independently associated with reduced CD4 gain (P <.001). A higher HIV RNA level also was associated with increases in activated CD8+CD38+ and CD8+HLA-DR+ cells (P <.001). A higher proviral DNA level was associated with increases in CD4+CD45RA−CD28− effector cells and reductions in naive CD4+CD45RA+CD62L+ and CD8+CD45RA+CD62L+ cells (P <.05). Higher levels of activated CD4+HLA-DR+ and early differentiated CD4+CD45RA+CD28+ cells predicted increased risk of subsequent detectable viremia in patients with undetectable HIV RNA (P <.05).

Conclusion. These findings indicate that low-level viremia and proviral DNA are intimately associated with the immunological and virological equilibrium in patients receiving HAART.
Table 1. Baseline characteristics and history of antiretroviral treatment for all 101 HIV-1–infected patients and for patients stratified according to HIV RNA level during the study period.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 101)</th>
<th>uVL patientsa (n = 33)</th>
<th>dVL patientsa (n = 68)</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at inclusion in study, years</td>
<td>44 (25–67)</td>
<td>44 (24–65)</td>
<td>45 (29–67)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, no. of male/no. of female patients</td>
<td>93/8</td>
<td>30/3</td>
<td>63/5</td>
<td>NS</td>
</tr>
<tr>
<td>Immunological and virological, at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of HIV infection at initiation of HAART, years</td>
<td>6 (0–16)</td>
<td>9 (0–13)</td>
<td>6 (0–16)</td>
<td>NS</td>
</tr>
<tr>
<td>AIDS diagnosis at inclusion in study, no. (%) of patients</td>
<td>25 (25)</td>
<td>8 (24)</td>
<td>17 (25)</td>
<td>NS</td>
</tr>
<tr>
<td>Pre-HAART CD4 cell count, cells/μL</td>
<td>170 (1–480)</td>
<td>180 (1–480)</td>
<td>170 (1–440)</td>
<td>NS</td>
</tr>
<tr>
<td>CD8 cell count, cells/μL</td>
<td>710 (110–3,800)</td>
<td>580 (110–1,700)</td>
<td>740 (110–3,800)</td>
<td>.048</td>
</tr>
<tr>
<td>HIV RNA level, copies/mL</td>
<td>65,722 (20–1,632,104)</td>
<td>22,764 (20–1,293,627)</td>
<td>95,458 (20–1,632,104)</td>
<td>NS</td>
</tr>
<tr>
<td>HIV RNA level at inclusion in study, no. (%) of patientsc</td>
<td></td>
<td>!.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UD</td>
<td>50 (50)</td>
<td>24 (73)</td>
<td>26 (38)</td>
<td></td>
</tr>
<tr>
<td>D-NQ</td>
<td>29 (29)</td>
<td>9 (27)</td>
<td>20 (29)</td>
<td></td>
</tr>
<tr>
<td>21–200 copies/mL</td>
<td>22 (21)</td>
<td>0</td>
<td>22 (33)</td>
<td></td>
</tr>
<tr>
<td>Treatment at inclusion in study and during follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAART before inclusion, months</td>
<td>14 (4–32)</td>
<td>14 (4–31)</td>
<td>14 (5–32)</td>
<td>NS</td>
</tr>
<tr>
<td>HIV RNA level ≤200 copies/mL at inclusion, months</td>
<td>9 (0–24)</td>
<td>9 (0–24)</td>
<td>6 (0–18)</td>
<td>6 (0–18)</td>
</tr>
<tr>
<td>PI-based HAART, no. (%) of patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Drugs</td>
<td>82 (81)</td>
<td>28 (85)</td>
<td>54 (79)d</td>
<td>NS</td>
</tr>
<tr>
<td>4 Drugs</td>
<td>18 (19)</td>
<td>5 (15)</td>
<td>13 (19)d</td>
<td>NS</td>
</tr>
<tr>
<td>NNRTI, no. (%) of patients</td>
<td>6 (6)</td>
<td>3 (9)</td>
<td>3 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>HAART modified during follow-up, no. (%) of patientsd</td>
<td>37 (37)</td>
<td>12 (36)</td>
<td>25 (37)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. Data are median (range), except where indicated otherwise. HAART, highly active antiretroviral therapy; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NS, not significant; PI, protease inhibitor.

a Patients were stratified into 2 groups according to HIV RNA level during the 24-month study period. uVL patients had undetectable HIV RNA levels at all time points. dVL patients had ≥1 episode of detectable viremia.

b Patient groups were compared by use of the 2-sample t test or by use of the χ² and Fisher’s exact tests. P values >.10 were NS and are not shown.

c Baseline HIV RNA samples analyzed by a standard assay (lower limit of detection, 200 copies/mL) were reanalyzed by an ultrasensitive assay (lower limit of detection, 20 copies/mL). Undetectable (UD) was given a value of 19 copies/mL, and detectable but nonquantifiable (D-NQ) was given a value of 20 copies/mL, for the statistical analysis (see Patients and Methods).

d One patient received only 2 NRTIs, but, since the statistical analyses excluding or including this patient had similar results, results for all patients are given.

e HAART was modified to another NRTI or PI combination or to include an NNRTI (2 uVL patients and 7 dVL patients).

ure. However, since most patients maintained good virological control during follow-up, the data obtained from this cohort were used to identify markers capable of predicting low-level viremia. Furthermore, data from this cohort were used to investigate associations between low-level viremia, proviral DNA, CD4 gain, and T cell subsets in patients receiving HAART.

PATIENTS AND METHODS

Study population. The present study was done at the Department of Infectious Diseases, Rigshospitalet (Copenhagen, Denmark), as described elsewhere [16]. During the period September 1997–August 1998, patients with reproducible plasma HIV RNA levels ≤200 copies/mL were included in the study. The patients were followed prospectively for 24 months: plasma HIV RNA level, CD4 cell count, and CD8 cell count were analyzed every 3 months, whereas T cell subsets and proviral DNA were analyzed every 6 months. Immunological and virological baseline data and history of antiretroviral treatment were extracted from patient files (table 1). The present study was based on results for 101 patients for whom flow-cytometric data at ≥2 time points were available; consequently, 2 patients from the cohort, described elsewhere [16], were excluded before data analysis. Written, informed consent was obtained from all subjects, and the study was approved by the local ethics committee for the Copenhagen and Frederiksberg Communities, Denmark (project 01-192/97).

Plasma HIV RNA and proviral DNA levels. Plasma HIV RNA was quantified by a standardized reverse-transcriptase polymerase chain reaction assay (Amplicor HIV-1 Monitor; Roche Diagnostic Systems). Some baseline samples were analyzed by...
a standard assay (lower limit of detection [LLD], 200 copies/mL), whereas all later samples were analyzed by an ultrasensitive assay (LLD, 20 copies/mL). Samples analyzed by the standard assay were reanalyzed by the ultrasensitive assay. Samples yielding a signal more than twice that of the mean from numerous negative control samples were recorded as detectable but non-quantifiable, whereas samples with weaker signals were recorded as undetectable. For statistical analysis, the samples were given values of 20 and 19 copies/mL, respectively, as described elsewhere [16]. Proviral HIV DNA copies per 10^9 peripheral blood mononuclear cells were quantified by a prototype assay (Amplicor HIV DNA assay; Roche Diagnostic Systems), in accordance with the manufacturer’s recommendations, as described elsewhere [16].

Flow-cytometric analysis of T cells. The total concentration of CD4 and CD8 T lymphocytes (×10^9 cells/L) were quantified with BD Triset CD4/CD8/CD3 TruCount Tubes (Becton Dickinson), by use of an Epics XL-MCL flow cytometer (Beckman Coulter). T cell subsets were analyzed in fresh whole blood, at inclusion of patients in the study and every 6 months for 24 months, by direct cell-surface 4-color immunofluorescent staining using anti-CD45, -CD14, -CD3, -CD4, -CD8, -CD45RA, -CD28, and -CD38 and IgG1 (Beckman Coulter) and anti–HLA-DR and IgG1 (DAKO). Labeled cells were analyzed by use of an Epics XL-MCL flow cytometer, and the subsequent computer analyses were done with WinList 4.0 (Verity Software House). Lymphocyte gates in forward/right-angle light scatter with <3% CD14+ monocytes were used in all analyses. The absolute cell concentration (×10^9 cells/L) was calculated by multiplying the proportion with the concentration of lymphocytes, obtained from hematological analysis. The following combinations of labeled antibodies were used (fluorescein isothiocyanate/phycoerythrin/R-phycoerythrin-cyanin-5.1/phycoerythrin–Texas red): CD45/CD14/-, CD62L/CD45RA/CD4/CD3, CD62L/CD45RA/CD8/CD3, CD28/CD45RA/CD4/CD3, HLA-DR/-/CD4/CD3, and HLA-DR/CD38/CD8/CD3. The following cell definitions were used [17–20]: naive CD4 or CD8 cells (CD45RA/CD62L·), memory CD4 or CD8 cells (CD45RA·CD45RO·), early differentiated CD4 cells (CD45RA·CD28·), late-differentiated CD4 cells (CD45RA·CD28·), effector CD4 cells (CD45RA·CD28·), and activated CD4 or CD8 cells (CD38· and/or HLA-DR·).

Statistics. Repeated-measures analyses for each investigated variable were done by use of a means model (the PROC MIXED model in the SAS software package), under the assumption of a first-order autoregressive covariance structure among the repeated measurements. Since the patients had been receiving HAART for various periods of time at inclusion, the timescale used was months from initiation of HAART. Group effects and time × group effects were included in the model, to investigate differences in the longitudinal changes between patients with and those without detectable HIV RNA during follow-up. Specific changes over time were analyzed by use of Bonferroni-adjusted, post hoc, paired t tests. Baseline variables for the 2 patient groups were compared by use of a 2-sample t test or by use of χ^2 and Fisher’s exact tests. The goodness of fit of the mixed model was assessed by investigation of the residuals.

A random-effects model (the PROC MIXED model in the SAS software package) that assumed a variance-component covariance structure and random effects between subject levels [21] was used to investigate the statistical association between the level of an explanatory variable and the dependent variable, during follow-up. For each patient, data obtained at all 5 time points (including missing values) contributed to the analysis.

By means of this model, we first investigated whether the levels of HIV RNA or proviral DNA both at baseline and during follow-up were associated with changes in the proportions of the investigated T cell subsets (table 2). Results are presented as the mean relative change in the proportion of a T cell subset during follow-up that was associated with a 10-fold increase in HIV RNA level or a 2-fold increase in proviral DNA level.

Next, we investigated whether the levels of specific T cell subsets both at baseline and during follow-up were associated with changes in CD4 cell count (i.e., CD4 gain) (table 3). Results are presented as the mean relative change in CD4 gain during follow-up that was associated with a 2-fold increase in the proportion of a T cell subset. To adjust for baseline variables with a known or potential effect on immune reconstitution, we investigated whether CD4 gain was affected by various baseline variables (age, AIDS diagnosis, pre-HAART CD4 and CD8 cell counts, pre-HAART HIV RNA level, preinclusion CD4 and CD8 gains per month, preinclusion decrease in HIV RNA level, treatment status, number of years HIV antibody positive at inclusion, or months of HAART before inclusion). A multivariate random-effects model, adjusted for significant univariate baseline variables, was subsequently fitted (table 3). All results from the random-effects model are presented with 95% confidence intervals (CIs). Goodness of fit was assessed by the plotting of residuals against predicted values, simultaneously for all data as well as separately for each subject.

A generalized linear model (the PROC GENMOD model in the SAS software package, repeated covariance structure) with a binomial response variable and a logit link function [22] was used to investigate whether a higher level of an explanatory variable at a given time point could predict the risk of subsequent detectable viremia (HIV RNA level >20 copies/mL [yes or no]) at the subsequent 3-month visit. To predict detectable viremia in patients with currently undetectable HIV RNA, the model only included data from time points when HIV RNA was undetectable. Results are presented as the odds ratio (OR),
Table 2. Associations between higher HIV RNA or proviral DNA level and proportions of specific T cell subsets in 101 HAART-treated, HIV-1–infected patients followed prospectively for 24 months.

<table>
<thead>
<tr>
<th>T cell subset</th>
<th>Relative change associated with Higher HIV RNA level</th>
<th>Relative change associated with Higher proviral DNA level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td></td>
<td>$^{P_a}$</td>
<td>$^{P_a}$</td>
</tr>
<tr>
<td>CD3$^+$CD4$^+$</td>
<td>1.06 (0.96–1.16) NS</td>
<td>0.94 (0.90–0.99) .013</td>
</tr>
<tr>
<td>CD4$^{5+}$CD62L$^+$ (naive)</td>
<td>1.02 (0.96–1.09) NS</td>
<td>0.99 (0.95–1.02) NS</td>
</tr>
<tr>
<td>CD4$^{5+}$CD28$^+$ (early differentiated)</td>
<td>0.94 (0.89–1.01) .083</td>
<td>1.02 (0.99–1.05) NS</td>
</tr>
<tr>
<td>CD4$^{5+}$CD28$^+$ (late differentiated)</td>
<td>1.04 (0.81–1.33) NS</td>
<td>0.96 (0.86–1.07) NS</td>
</tr>
<tr>
<td>CD4$^{5+}$CD28$^+$ (effector)</td>
<td>1.04 (0.73–1.48) NS</td>
<td>1.24 (1.01–1.52) .041</td>
</tr>
<tr>
<td>HLA-DR$^+$ (activated)</td>
<td>1.06 (0.93–1.19) NS</td>
<td>0.97 (0.91–1.02) NS</td>
</tr>
<tr>
<td>CD3$^+$CD8$^+$</td>
<td>1.02 (0.92–1.13) NS</td>
<td>0.93 (0.89–0.98) .007</td>
</tr>
<tr>
<td>CD4$^{5+}$CD45RO$^+$ (memory)</td>
<td>1.09 (1.02–1.17) .017</td>
<td>0.99 (0.96–1.02) NS</td>
</tr>
<tr>
<td>CD38$^+$HLA-DR$^+$ (activated)</td>
<td>1.22 (1.08–1.37) .002</td>
<td>0.96 (0.91–1.02) NS</td>
</tr>
<tr>
<td>HLA-DR$^+$ (activated)</td>
<td>1.11 (1.02–1.22) .015</td>
<td>0.97 (0.93–1.02) NS</td>
</tr>
<tr>
<td>CD38$^+$ (activated)</td>
<td>1.11 (1.01–1.22) .032</td>
<td>0.97 (0.93–1.02) NS</td>
</tr>
</tbody>
</table>

NOTE. A random-effects model (the PROC MIXED model in the SAS software package) was used to estimate the statistical association between the level of HIV RNA (10-fold higher) or proviral DNA (2-fold higher) and the mean relative change in the proportion of the investigated T cell subsets during follow-up. For the analysis of relative change, values $<1$ or $>1$ were interpreted as a relative reduction or increase, respectively, in the proportion of T cell subsets, and a value equal to 1 was interpreted as no change. CI, confidence interval; HAART, highly active antiretroviral therapy; NS, not significant.

$^{P_a}$ P values $>0.10$ were NS and are not shown.

with 95% CI, for detectable viremia at the subsequent 3-month visit, as predicted by a 2-fold increase in the proportion of a T cell subset. Goodness of fit was assessed by the plotting of grouped explanatory variables against the logit function and by inclusion of continuous and grouped explanatory variables in the same model.

Data are presented as medians with ranges or interquartile ranges (IQRs). $P<.05$ was considered to be significant. Statistical calculations were performed by use of SAS 8.2 (SAS Institute).

RESULTS

Baseline characteristics and study groups. A total of 101 HAART-treated patients who both fulfilled the HIV RNA inclusion criteria and had available flow-cytometric data consented to participate (table 1), as described elsewhere [16]. Changes in virological and immunological parameters after initiation of HAART, the number of samples from patients, and the number and proportion of patients with undetectable HIV RNA during follow-up are shown in figure 1.

The patients were stratified according to HIV RNA level during the study period: 33 patients had HIV RNA levels $\leq$20 copies/mL at all visits (uVL patients), whereas 68 patients had HIV RNA levels $>20$ copies/mL at $\geq1$ visit (dVL patients) (table 1). Only dVL patients had HIV RNA levels of 21–200 copies/mL at inclusion (table 1). During follow-up, HIV RNA and proviral DNA levels were higher in the dVL patients (group effect $P<.001$ and $P = .003$, respectively), and HIV RNA levels increased in the dVL patients ($P<.001$), whereas proviral DNA levels remained unchanged in both groups (data not shown). The increase in HIV RNA levels at time points when virological rebound occurred in the dVL patients was modest, with a median increase of 81 HIV RNA copies/mL (IQR, 37–480 HIV RNA copies/mL). Only 4 patients had $>2$ consecutive episodes with $>10,000$ HIV RNA copies/mL. In comparisons of baseline characteristics between the uVL and the dVL patients, pre-HAART CD8 cell count was higher among the dVL patients. No other baseline characteristics differed between the 2 groups (table 1).

Antiretroviral treatment. The patients had received HAART for a median period of 14 months (range, 4–32 months) at inclusion (table 1). Most patients had received protease inhibitor (PI)–based therapy with 3 or 4 drugs, and some patients also had received a nonnucleoside reverse-transcriptase inhibitor (NNRTI) (table 1). One patient had received 2 nucleoside reverse-transcriptase inhibitors (NRTIs) at inclusion, but, since similar results were obtained whether the statistical analyses excluded or included this patient, results are shown for all patients. No patients discontinued HAART during follow-up, but 37 patients had their antiretroviral treatment modified.
Table 3. Associations between higher HIV load or higher proportions of specific T cell subsets and CD4 gain in 101 HAART-treated, HIV-1–infected patients followed prospectively for 24 months.

<table>
<thead>
<tr>
<th>Type of HIV load or T cell subset</th>
<th>Relative change in CD4 gain</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>P*</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>HIV RNA</td>
<td>0.72 (0.61–0.85)</td>
<td>&lt;.001</td>
<td>0.69 (0.58–0.82)</td>
</tr>
<tr>
<td>Proviral DNA</td>
<td>1.00 (0.97–1.03)</td>
<td>NS</td>
<td>0.98 (0.83–1.16)</td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45RA+CD62L+ (naive)</td>
<td>1.14 (1.09–1.18)</td>
<td>&lt;.001</td>
<td>1.13 (1.09–1.18)</td>
</tr>
<tr>
<td>CD45RA+CD45RO+ (memory)</td>
<td>0.92 (0.86–0.99)</td>
<td>.027</td>
<td>0.94 (0.88–1.01)</td>
</tr>
<tr>
<td>CD45RA+CD28+ (early differentiated)</td>
<td>0.93 (0.88–0.99)</td>
<td>.029</td>
<td>0.98 (0.92–1.04)</td>
</tr>
<tr>
<td>CD45RA+CD28+ (late differentiated)</td>
<td>1.00 (0.98–1.02)</td>
<td>NS</td>
<td>0.99 (0.96–1.01)</td>
</tr>
<tr>
<td>CD45RA+CD28+ (effector)</td>
<td>1.00 (0.99–1.01)</td>
<td>NS</td>
<td>1.00 (0.99–1.01)</td>
</tr>
<tr>
<td>HLA-DR+ (activated)</td>
<td>0.98 (0.96–1.01)</td>
<td>NS</td>
<td>0.99 (0.95–1.02)</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45RA+CD62L+ (naive)</td>
<td>1.08 (1.04–1.12)</td>
<td>&lt;.001</td>
<td>1.09 (1.05–1.13)</td>
</tr>
<tr>
<td>CD45RA+CD45RO+ (memory)</td>
<td>1.00 (0.94–1.06)</td>
<td>NS</td>
<td>1.03 (0.97–1.09)</td>
</tr>
<tr>
<td>CD38+HLA-DR+ (activated)</td>
<td>0.97 (0.94–1.00)</td>
<td>.029</td>
<td>0.97 (0.94–1.00)</td>
</tr>
<tr>
<td>HLA-DR+ (activated)</td>
<td>1.00 (0.96–1.04)</td>
<td>NS</td>
<td>1.02 (0.97–1.07)</td>
</tr>
<tr>
<td>CD38+ (activated)</td>
<td>0.95 (0.91–0.99)</td>
<td>.013</td>
<td>0.96 (0.92–1.00)</td>
</tr>
</tbody>
</table>

**NOTE.** A random-effects model (the PROC MIXED model in the SAS software package) was used to estimate the statistical association between the level of HIV RNA (10-fold higher) or proviral DNA (2-fold higher) or the proportion of T cell subsets (2-fold higher) and the mean relative change in CD4 gain during follow-up, in univariate and multivariate models adjusted for pre-HAART CD4 and CD8 cell counts, preinclusion CD4 gain (10 cells/μL/month), and previous AIDS diagnosis. For the analysis of relative change, values <1 or >1 were interpreted as a relative reduction or increase, respectively, in CD4 gain, and a value equal to 1 was interpreted as no change. CI, confidence interval; HAART, highly active antiretroviral therapy; NS, not significant.

* P values >.10 were NS and are not shown.

either to another NRTI or PI combination or to include an NNRTI (table 1). For the majority of patients, the modification of therapy was due to adverse effects, rather than to treatment failure. After modification of HAART, the patients were still included in the statistical analyses.

Initial antiretroviral therapy before initiation of HAART consisted of 1, 2, 3, or 4 drugs in 38, 24, 34, and 5 patients, as described elsewhere [16]. The study groups had comparable treatment history and treatment regimens during follow-up (table 1).

**Longitudinal changes in T cell subsets in uVL and dVL patients.** Owing to the low number of subjects included in the period outside 12–36 months after initiation of HAART (figure 1), the longitudinal changes in T cell subsets were only investigated during this period (figure 2C–2H). The HAART-induced changes in CD4 and CD8 cell counts were equal in the uVL and the dVL patients (figure 2A and 2B), although CD8 cell count was higher in the dVL patients (figure 2B). The concentrations of naive CD4 cells, naive CD8 cells, and activated CD4 cells increased equally in the 2 groups (figure 2C, 2D, and 2G), although the concentration of activated CD4 cells was higher in the dVL patients (figure 2G). The concentrations of memory CD4 cells, memory CD8 cells, and activated CD8 cells did not change during follow-up, but they were higher in the dVL patients (figure 2E, 2F, and 2H).

**Association between HIV RNA or proviral DNA level and T cell subsets.** A 10-fold higher HIV RNA level during follow-up was associated with a 9% increase in the proportion of memory CD8 cells (table 2); that is, the mean change in memory CD8 cells in all patients during follow-up was 9% per 10-fold increase in HIV RNA level (depicted in figure 2F, where the proportion of memory CD8 cells in the dVL patients appears to have increased slightly, compared with that in the uVL patients). In addition to an increase in the proportion of memory CD8 cells, higher HIV RNA level also was associated with an increase in the proportion of activated CD8 cells (table 2).

A higher proviral DNA level was associated with reductions in the proportions of naive CD4 and CD8 cells (table 2). Conversely, a higher proviral DNA level was associated with an increase in the proportion of effector CD4 cells (table 2). Similar results were found for concentrations of the investigated T cell subsets (data not shown).

**Association between HIV RNA level or T cell subsets and CD4 gain.** In accordance with previous findings [23, 24], previous AIDS diagnosis, pre-HAART CD4 and CD8 cell counts, and preinclusion CD4 gain were found to affect CD4
gain during follow-up, in the univariate analysis (data not shown). Consequently, these variables were included in the multivariate model (table 3).

A 10-fold increase in HIV RNA level during follow-up was independently associated with a 31% reduction in CD4 gain, whereas proviral DNA level had no effect on CD4 gain (table 3). Furthermore, a 2-fold increase in the percentage of total CD8 cells was independently associated with a 10% reduction in CD4 gain (relative change, 0.90; 95% CI, 0.83–0.98; \( P < .001 \)).

Higher proportions of naive CD4 and CD8 cells were independently associated with increases in CD4 gain, whereas a higher proportion of activated CD8 cells was independently associated with a reduction in CD4 gain (table 3). Higher proportions of memory CD4 cells, early differentiated CD4 cells, and activated CD8 cells were associated with reductions in CD4 gain, in the univariate analysis (table 3).

**Risk of subsequent detectable viremia predicted by increases in specific T cell subsets.** We investigated whether a higher proportion of a specific T cell subset at a given time point could predict the risk of detectable viremia at the subsequent 3-month visit. Since none of the investigated baseline variables (mentioned in the “Statistics” subsection of Patients and Methods) could predict subsequent viremia, only a univariate model was fitted.

A 2-fold increase in the proportion of activated CD4 cells predicted a 39% increased risk of subsequent detectable viremia (table 4). Furthermore, a higher proportion of early differentiated CD4 cells and late-differentiated CD4 cells predicted increased and reduced risk of subsequent detectable viremia, respectively (table 4). Similar results were found when concentrations of T cells were used to predict subsequent detectable viremia (data not shown).

**DISCUSSION**

The present study investigated the associations between low-level viremia, proviral DNA, CD4 gain, and T cell subsets in HIV-1–infected patients with undetectable HIV RNA after initiation of HAART. The following were new findings: (1) even low-level viremia was associated with a reduction in CD4 gain; (2) proviral DNA level was associated with CD4 T cell activation; (3) CD4 T cell activation predicted subsequent viremia; and (4) early and late-differentiated CD4 cells had opposite effects on the risk of subsequent viremia.

First, in the present study, a higher HIV RNA level was independently associated with a reduction in CD4 gain, as well as with increases in the proportions of memory and activated CD8 cells. Since a large majority of the patients maintained relatively good virological control during follow-up (median increase in HIV RNA level at time points with virological rebound, 81 copies/mL [IQR, 37–480 copies/mL]), these findings can most likely be attributed to low-level viremia.

It recently has been demonstrated that viral replication might continue in patients with undetectable HIV RNA [25, 26] and that intensification of HAART for these patients leads to significant reductions in viral replication [27, 28] and T cell activation [27]. In contrast to the results of the present study, Havlir et al. [27] found no association between low-level viremia and CD4

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**Figure 1.** Concentrations of CD4 cells, CD8 cells, and plasma HIV RNA from initiation of highly active antiretroviral therapy (HAART) to 54 months afterward, in 101 HIV-1–infected patients. Medians and interquartile ranges are shown. The patients were included after plasma HIV RNA level was found to be undetectable, and each patient was followed prospectively for 24 months. The median period from initiation of HAART to inclusion in the study was 14 months (range, 4–32 months). The no. of patient samples and the no. and proportion of patients with undetectable plasma HIV RNA (≤20 copies/mL) are indicated.
Concentrations of CD4 cells, CD8 cells, and various T cell subsets in patients stratified according to plasma HIV RNA level during the study period. Thirty-three patients had HIV RNA levels ≤20 copies/mL at all visits (undetectable HIV RNA [uVL patients]), whereas 68 patients had HIV RNA levels >20 copies/mL at ≥1 visit (detectable viremia [dVL patients]). The medians and interquartile ranges are shown. The total concentrations of CD4 and CD8 cells are shown from the initiation of highly active antiretroviral therapy (HAART) to 36 months afterward (a and b), whereas the concentrations of naive, memory, and activated CD4 and CD8 cells are shown for the period 12–36 months after initiation of HAART (c–h), owing to the low no. of subjects contributing to the analysis outside this period. The total concentrations of CD4 cells (a), CD8 cells (b), naive CD4+CD45RA+CD62L+ cells (c), naive CD8+CD45RA+CD62L+ cells (d), memory CD4+CD45RA+CD45RO+ cells (e), memory CD8+CD45RA+CD45RO+ cells (f), activated CD4+HLA-DR+ cells (g), and activated CD8+CD38+HLA-DR+ cells (h) are shown. P values for time, group, and time x group effects in the mixed repeated models are shown. Significant differences between time point 0 (a and e) or 12 (b–d and f–h) and the subsequent time points were analyzed for all patients (combining uVL and dVL patients, since time x group effects were not significant [NS]), by means of Bonferroni-adjusted, post hoc, paired t tests: *P < .05; **P < .01; ***P < .001. ANOVA, analysis of variance.
CD8 cells and in proviral DNA level were comparable in uVL.

In the present study, the changes in the concentration of total CD4 cells and naive CD4 and CD8 cells increased equally in uVL and dVL patients 12–36 months after initiation of HAART. Since most of the dVL patients receiving HAART are negatively associated with immune reconstitution, either directly or through induction of immune activation.

Second, a higher proviral DNA level was associated with an increase in the proportion of effector CD4 cells and with reductions in the proportions of naive CD4 and CD8 cells. Whether this finding can be attributed to replication-competent or replication-defective genomes or to both, through production of viral proteins, is not known, but this finding indicates that the proviral DNA level interferes with immune reconstitution in patients receiving HAART. Further studies are needed to confirm this finding and to understand the potential biological effect of proviral DNA in patients receiving HAART.

Third, the present study investigated whether a higher proportion of specific T cell subsets could predict the risk of detectable viremia, at the subsequent 3-month visit, in patients with undetectable HIV RNA. Higher proportions of activated CD4 and CD8 cells predicted an increased risk of subsequent detectable viremia. The finding that the proportion of activated CD8 cells could not predict subsequent detectable viremia may seem notable but most likely reflects that activation of HIV-infected CD4 cells leads to HIV replication. Although the CD4 T cell activation detected in the present study may be attributed to both low-level viral replication and concurrent infections, this finding suggests that CD4 T cell activation favors viral replication even during HAART.

Fourth, we found that higher proportions of early or late-differentiated CD4 cells predicted an increased or a reduced risk, respectively, of subsequent detectable viremia. Different functions of early and late-differentiated CD4 cells may explain this finding. In brief, early differentiated CD4 cells have a high proliferative capacity but no cytotoxic potential, whereas late-differentiated CD4 cells have a reduced proliferative capacity but a cytotoxic potential [19]. Thus, cytotoxic late-differentiated CD4 cells possibly exhibit cytotoxicity against HIV-infected cells. However, the opposite predictive value of early and late-differentiated CD4 cells also could reflect a high or a low degree of HIV infection, respectively, for these cells.

**Table 4. Risk of subsequent viremia predicted by higher proportions of specific T cell subsets in 101 HAART-treated, HIV-1-infected patients with undetectable HIV RNA who were followed prospectively for 24 months.**

<table>
<thead>
<tr>
<th>T cell subset</th>
<th>Risk of subsequent viremia, OR (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>CD45RA+CD62L+ (naive)</td>
<td>0.98 (0.75–1.26)</td>
<td>NS</td>
</tr>
<tr>
<td>CD45RA+CD45RO+ (memory)</td>
<td>1.36 (0.71–2.6)</td>
<td>NS</td>
</tr>
<tr>
<td>CD45RA+CD28+ (early differentiated)</td>
<td>2.18 (1.05–4.51)</td>
<td>.037</td>
</tr>
<tr>
<td>CD45RA+CD28+ (late differentiated)</td>
<td>0.76 (0.59–0.98)</td>
<td>.036</td>
</tr>
<tr>
<td>CD45RA+CD28+ (effector)</td>
<td>1.06 (0.88–1.26)</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-DR+ (activated)</td>
<td>1.39 (1.06–1.82)</td>
<td>.018</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>CD45RA+CD62L+ (naive)</td>
<td>0.94 (0.71–1.25)</td>
<td>NS</td>
</tr>
<tr>
<td>CD45RA+CD45RO+ (memory)</td>
<td>1.04 (0.68–1.59)</td>
<td>NS</td>
</tr>
<tr>
<td>CD38+HLA-DR+ (activated)</td>
<td>1.20 (0.92–1.58)</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-DR+ (activated)</td>
<td>1.12 (0.77–1.63)</td>
<td>NS</td>
</tr>
<tr>
<td>CD38+ (activated)</td>
<td>1.12 (0.74–1.69)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** A generalized linear model (the PROC GENMOD model in the SAS software package) was used to investigate whether a 2-fold higher proportion of the investigated T cell subsets, at a given time point, could predict the risk of detectable viremia at the subsequent 3-month visit, for patients with undetectable HIV RNA. Detectable viremia was defined as HIV RNA >20 copies/mL. CI, confidence interval; HAART, highly active antiretroviral therapy; NS, not significant; OR, odds ratio.

*P values >.10 were NS and are not shown.*

gain in patients receiving HAART. However, the divergent findings with regard to CD4 gain could be explained by differences in the magnitude of the low-level viremia (<23 HIV RNA copies/mL in [27]) or by the low number of subjects (n = 5) included in the study by Havlir et al. [27]. Although the present study is the first, to our knowledge, to demonstrate a negative association between even low-level viremia and immune reconstitution in patients receiving HAART, this potentially important result should be confirmed in later studies.

The concentration of total CD4 cells and naive CD4 and CD8 cells increased equally in uVL and dVL patients 12–36 months after initiation of HAART. Since most of the dVL patients had intermittent viremia, the divergent findings in the present study could indicate that CD4 gain was reduced at time points when viremia was detectable but not at time points when HIV RNA was undetectable (the random-effects model estimated the association between a higher HIV RNA level [i.e., detectable] and CD4 gain, at individual time points). This notion—that is, time points when even low-level viremia was detected may have been associated with altered immune function—is supported by a recent study demonstrating varying immune function at time points when viremia was or was not detectable, in patients with intermittent low-level viremia [7].

In the present study, the changes in the concentration of total CD8 cells, activated CD8 cells, memory CD4 cells, and memory CD8 cells and in proviral DNA level were comparable in uVL and dVL patients. However, these variables were higher in the dVL patients, indicating that low-level viremia may be associated with T cell activation and a higher proviral DNA level. The present study also found a negative association between T cell activation and CD4 gain. This finding is in accordance with a recent study, by Hunt et al. [29], demonstrating that higher levels of activated CD4 and CD8 cells in HAART-treated patients are associated with reduced early and reduced late CD4 gains, respectively. The negative effect of T cell activation on the progression of HIV disease and on CD4 gain in untreated and HAART-treated patients, respectively, most likely reflects that T cell depletion in HIV infection is driven mainly by immune activation [30–34]. Overall, the findings in the present study indicate that periods of even low-level viremia in patients receiving HAART are negatively associated with immune reconstitution, either directly or through induction of immune activation.

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When interpreting the findings in the present study, some limitations should be considered. (1) The study was designed to investigate virological and immunological markers capable of predicting virological failure in HAART-treated patients. However, since most patients maintained good virological control during follow-up, the data from this cohort were used to identify markers capable of predicting low-level viremia. Thus, the results from the present study should be confirmed by other studies. (2) The patients were stratified according to HIV RNA level during follow-up (detectable viremia vs. undetectable HIV RNA), without distinction between patients with intermittent and those with persistent low-level viremia. However, the present study did investigate whether the longitudinal changes in the investigated T cell subsets and proviral DNA differed between patients with intermittent (detectable viremia at ≤50% of the time points \( n = 47 \)) and those with persistent (detectable viremia at >50% of the time points \( n = 21 \)) low-level viremia, but comparable results were found for these groups (data not shown). (3) The random-effects model used in the present study had some limitations. Since the investigated virological and immunological variables were measured concurrently, cause-and-effect inferences simply cannot be made. Thus, the finding that, for example, a higher level of T cell activation was associated with a reduction in CD4 gain also could reflect that persistent immunodeficiency with a low CD4 cell count results in persistent T cell activation, owing to the presence of concurrent infections. Furthermore, since the patients had been receiving HAART for varying times at inclusion, the estimated changes during follow-up represent an mean of early (larger) and late (smaller) changes in the investigated parameters. Finally, although several markers were investigated, \( P \) values were not adjusted for multiple comparisons, which further strengthens the need for confirmation of the results in studies of independent cohorts.

In summary, the present study found that even low-level viremia was associated with immunological and virological consequences in patients receiving HAART. Although low-level viremia did not result in obvious treatment failure, it was associated with reductions in CD4 gain and with T cell activation. Furthermore, CD4 T cell activation predicted subsequent increases in HIV replication even during HAART. Finally, a higher proviral DNA level was associated with CD4 T cell activation and reductions in naive CD4 and CD8 cells, indicating an immunological effect of proviral DNA even in patients with sustained virological suppression. It is not known to what degree persistent immune activation and immunological dysregulation accounts for the emergence of, for example, metabolic abnormalities and putative HIV-related dementia in patients receiving HAART. However, it is tempting to speculate whether further reductions in HIV replication and T cell activation through intensification of HAART may have a positive effect on immune reconstitution and certain long-term complications.

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References


