Down-Regulation of Interleukin-7 Receptor (CD127) in HIV Infection Is Associated with T Cell Activation and Is a Main Factor Influencing Restoration of CD4+ Cells after Antiretroviral Therapy

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Background. Factors influencing the depletion of CD4+ cells and the restoration of CD4+ cells after antiretroviral therapy are not completely understood. Recently, attention has been paid to interleukin (IL)–7 and its receptor (CD127). We analyzed the influence of T cell activation and of suppression of viremia with antiretroviral therapy on this system, as well as its role in CD4+ cell restoration after long-term antiretroviral therapy.

Methods. IL-7 levels and CD127 expression on several subsets of CD4+ and CD8+ T lymphocytes and the activation status (CD38) of these cells were examined at baseline and during 24 months of complete viral suppression under highly active antiretroviral therapy (HAART).

Results. A total of 42 individuals with human immunodeficiency virus (HIV) infection and 10 age-matched, uninfected control subjects were examined. Before HAART, IL-7 levels were increased and CD127 expression was decreased. Down-regulation of CD127 was mainly associated with T cell activation and reverted only partially after suppression of detectable plasma HIV RNA with HAART. In a multivariate analysis, CD127 expression on CD8+ T cells was the main determinant of the extent of CD4+ cell gains after successful HAART.

Conclusions. The IL-7–CD127 system is impaired in HIV-infected patients. CD127 down-regulation is associated with T cell activation and with CD4+ cell restoration after HAART.

HIV infection produces a progressive loss of and dysfunction among CD4+ T lymphocytes [1]. Several mechanisms are involved in the depletion of these cells: redistribution, destruction of infected cells, death of uninfected cells by apoptosis, and inability of progenitor cells in the bone marrow and thymocytes to provide adequate regeneration [2]. Immune abnormalities during HIV infection are not limited to depletion of and dysfunction among CD4+ T cells. An imbalance in the distribution of several lymphocyte subsets defined on the basis of distinct surface markers is a hallmark of HIV infection, including abnormalities in memory or naive subpopulations and activated cells [3–6]. Measurement of activation markers, such as CD38, may predict HIV disease progression independently of CD4+ cell counts and plasma viremia [7–9]. CD8+CD38+ T cell counts have occasionally been even more predictive of HIV disease progression than CD4+ cell counts or plasma viremia [10–12]. In most patients with HIV infection, highly active antiretroviral therapy (HAART) suppresses viral replication to undetectable levels, which is generally accompanied by an increase in the CD4+ cell population [13]. However, the extent of CD4+ cell reconstitution is highly variable, and several factors may influence increases in the CD4+ cell population during receipt of HAART [14]. Attention has recently been paid to the degree of immune activation [15, 16].

Interleukin (IL)–7 has been thought to be involved in the pathogenesis of CD4+ cell depletion and in the extent of CD4+ T cell reconstitution in HIV-infected patients after HAART initiation. IL-7 is a cytokine that plays a pivotal role in the homeostasis of T cells, promoting survival of naive T cells and generation of memory...
cells [17]. Serum IL-7 levels are increased in patients with chronic HIV infection, supposedly in response to reduced CD4+ T cell counts [18]. Accordingly, increases in CD4+ cell population after viral suppression with HAART are generally followed by decreases in serum IL-7 levels [19, 20]. Finally, an association has been noticed between serum IL-7 levels before the initiation of HAART and the extent of increase in the CD4+ cell population [20].

The IL-7 receptor is made up of a common γ-chain shared with other cytokines and a specific α-chain (CD127), and it is expressed on most T lymphocytes [21]. In patients with HIV infection, a reduced expression of CD127 on T cells is uniformly seen, particularly with CD8 T cells [22–25]. This finding has been associated with high IL-7 levels, suggesting a down-regulation that might render T cells unresponsive to IL-7 in HIV infection [22, 24, 25].

In this study, we examine the relationship between serum IL-7 levels, CD127 expression, and activation markers in different CD4+ and CD8+ T cell subsets in HIV-infected individuals. The evolution of these parameters after achievement of viral suppression with HAART and its association with the extent of increase in the CD4+ cell population are further examined.

PATIENTS AND METHODS

Study population. A group of 42 untreated patients with chronic HIV infection who subsequently underwent HAART and achieved plasma HIV RNA levels of <50 copies/mL for at least 24 months were identified at our institution. The mean CD4+ cell count and plasma HIV RNA level at baseline were 310 ± 208 cells/μL and 4.7 ± 0.57 log copies/mL, respectively. Ten age-matched, HIV-uninfected control subjects were recruited from among healthy laboratory personnel. Written informed consent for this study was obtained from all individuals, and the study protocol was approved by the hospital’s ethical committee.

Flow cytometry. The levels of different subsets of CD4+ and CD8+ T cells, CD127 expression, and immune activation (with CD38 used as a surrogate marker) were evaluated in peripheral blood mononuclear cells (PBMCs). CD45RA and CD27 were used to define the maturation status of CD4+ and CD8+ T cells. Accordingly, 4 subsets were defined: naive (CD45RA+CD27+), central memory (CD45RA-CD27+), effector memory (CD45RA-CD27−), and effector (CD45RA-CD27−).

CD38 expression in different T cell subsets was measured by using a 5-color, modified version of a commercial quantitative flow cytometry assay (Cellquant CD38; Biocytex) [16]. In combination with CD38, the next panel of monoclonal antibodies was used in the flow cytometer analysis: CD127–phycoerythrin (PE), CD45RA–ECO, CD27–PE-cyanine (Cy) 5 and CD4–PE-Cy7 or CD8–PE-Cy7 (Beckman Coulter). Staining was performed on previously frozen PBMCs, and a minimum of 5000 CD4+ or CD8bright cells per sample were examined using an FC 500 cytometer (Beckman Coulter).

Serum IL-7 level. Serum IL-7 level was measured by using a highly sensitive commercial immunoassay (Quantikine HS; R&D Systems), with a lower limit of detection of 0.1 pg/mL.

Plasma HIV RNA level. Plasma HIV RNA level was quantified by using the third-generation branched DNA assay (Quantiplex, version 3.0; Siemens Molecular Systems), with a lower detection limit of 50 copies/mL HIV RNA.

Statistical analyses. Medians and interquartile ranges (IQRs) were used to describe each of the variables analyzed. Differences between controls and HIV-infected patients or between different time points in patients receiving HAART were assessed using the Mann-Whitney nonparametric test. Associations between different variables were explored by using the Pearson correlation coefficient. A multivariate linear regression model was established to ascertain which baseline parameters had a greater influence on increases in CD4+ cell population after the beginning of HAART. All statistical analyses were performed using SPSS software (version 13.0; SPSS).

RESULTS

CD127 expression and activation in different T cell subsets in untreated, HIV-infected patients. The levels of CD127 and CD38 were evaluated in 4 subsets of CD4+ and CD8+ T cells defined by expression of CD45RA and CD27. With respect to CD4+ T lymphocytes, HIV-infected patients tended to show lower levels of naive and higher levels of effector memory cells than HIV-seronegative control subjects, although differences were not significant. The profile of CD127 expression was similar in HIV-infected patients and control subjects. In both groups, CD127 was higher in effector memory and central memory cells, followed first by naive cells and finally by effector cells. CD127 expression on total CD4+ cells was similar in HIV-infected patients and control subjects, although expression of CD127 on effector memory CD4+ cells was significantly decreased in HIV-infected patients (figure 1).

Differences between patients and control subjects were more pronounced for CD8+ T cell subsets. Effector cells were significantly increased in patients compared with control subjects (median [IQR], 32% [24%] vs. 10% [15%], respectively; P < .001). In contrast, naive cells were significantly decreased in HIV-infected patients compared with control subjects (median [IQR], 17% [16%] vs. 53% [29%), respectively; P < .001). The profile of CD127 expression on CD8+ T cell subsets was similar in HIV-infected patients and control subjects, with the higher level of expression seen in memory cells, followed first by naive cells and finally by effector cells. CD127 expression on total CD8+ T cells was significantly diminished in HIV-infected patients, particularly in the subset of effector cells (figure 1).
CD38 were measured in the 8 CD4\(^+\)/H11001 and CD8\(^+\)/H11001 T cell subsets defined by CD45RA, CD27, and CD127 markers (figure 2). In HIV-infected patients, activation was significantly increased on total CD4\(^+\)/H11001 T cells as well as in memory and effector cells not expressing CD127. CD4\(^+\)/H11001 T cell subsets expressing CD127 did not have significantly increased levels of activation, except for central memory cells, which showed a slight increase in CD38 expression. In HIV-seronegative control subjects, the highest activation levels were seen in central memory and naive T cell subsets not expressing CD127, and the lowest in central memory cells expressing CD127. In HIV-infected patients the profile of activation was different, with the highest level observed in central memory cells not expressing CD127 (as in control subjects), followed by effector memory and effector cells not expressing CD127 (figure 2).

CD38 expression was significantly increased in most CD8\(^+\)/H11001 T cell subsets of HIV-infected patients, especially in those not expressing CD127 except in naive cells (both CD127\(^+\) and CD127\(^-\)) and central memory cells not expressing CD127 (figure 2). The profile of CD38 expression was similar in HIV-infected patients and control subjects, with the highest level seen in central memory cells not expressing CD127 and the lowest in effector memory cells expressing CD127. As in CD4\(^+\)/H11001 T cells, the highest degree of increased activation in CD8\(^+\)/H11001 T cells from HIV-infected patients was seen in effector memory and effector cells not expressing CD127. In control subjects, the frequency of these subsets was very low because most CD8\(^+\)/H11001 T cells expressed CD127.

**Figure 2.** Levels of CD38 expression on different subsets of CD4\(^+\)/H11001 and CD8\(^+\)/H11001 cells (top graph) and CD8\(^+\)/H11001 cells (bottom graph) in untreated HIV-infected patients (open boxes) and seronegative control subjects (gray boxes). \(*\) P < .001 for comparison between patients and control subjects.

Associations between CD127 expression, immune activation, CD4\(^+\)/H11001 cell counts, and plasma HIV RNA and serum IL-7 levels in untreated patients. There was no significant correlation between IL-7 level and either plasma HIV RNA level or CD4\(^+\)/H11001 cell counts. However, in patients with CD4\(^+\) cell counts <500 cells/µL, a significant inverse correlation was seen between CD4\(^+\)/H11001 cell counts and IL-7 level after adjustment for viral load (r = −0.4; P = .02).

The association of CD4\(^+\)/H11001 cell count, plasma HIV RNA level, and IL-7 level with activation in different T cell subsets was examined. Activation of CD4\(^+\)/H11001 T cells was mainly associated with CD4\(^+\)/H11001 cell count (total population) and plasma viremia (effector memory T cell subset not expressing CD127) (figure 3). Activation of CD8\(^+\)/H11001 T cells was associated with plasma HIV RNA or IL-7 level, depending on the subset considered. Central memory cells (both CD127\(^-\) and CD127\(^+\)) showed the strongest correlation with plasma viremia, whereas effector cells (both CD127\(^-\) and CD127\(^+\)) mainly correlated with IL-7 level. CD4\(^+\)/H11001 cell count was also associated with activation of different CD8\(^+\)/H11001 T cell subsets, although to a lesser extent.

Finally, we tested the potential associations between level of CD127 expression and CD4\(^+\)/H11001 cell count, HIV load, IL-7 level, and activation level, using a multivariate analysis in which CD4\(^+\)/H11001 cell count, plasma viremia, IL-7 level, and activation were included as explanatory variables, and CD127 expression on different T cell subsets was the dependent variable (figure 4). CD127 expression on total CD4\(^+\)/H11001 T cells and central memory T
cells was inversely correlated with activation of CD4^+ T cells \((r = -0.48 \ [P = .002] \text{ and } r = -0.58 \ [P < .001], \text{ respectively})\). On the other hand, CD127 expression on effector memory CD4^+ T cells inversely correlated with plasma viremia \((r = -0.42; \ P = .007)\). In CD8^+ T cells, CD127 expression on the total population or in central memory, effector memory and effector T cell subsets was inversely correlated with activation of CD8^+ T cells \((r = -0.44 \ [P = .005], \ r = -0.57 \ [P < .001], \ r = -0.6 \ [P < .001], \text{ and } r = -0.39 \ [P = .02], \text{ respectively})\).

**Evolution of CD4^+ cell count, serum IL-7 level, and CD127 expression in HIV-infected patients receiving HAART.** CD4^+ cell counts significantly increased after 12 and 24 months of complete viral suppression with HAART \((310 \pm 208, 517 \pm 320, \text{ and } 576 \pm 339 \text{ cells/µL at months 0, 12, and 24, respectively; } P < .001)\), whereas serum IL-7 level tended to decrease after 24 months of therapy, although this difference did not reach statistical significance \((4.4 \pm 4.2 \text{ and } 3.1 \pm 2.6 \text{ pg/mL at months 0 and 24, respectively; } P = .1)\).

CD127 expression did not significantly change during HAART in most CD4^+ T cell subsets, except for central memory cells, which showed a significant increase at month 24 of treatment. The low level of CD127 expression on effector memory CD4^+ T cells at month 0 was still present after 24 months of HAART. In contrast, CD127 expression significantly increased in most CD8^+ T cell subsets by month 12 of HAART and continued to increase thereafter (figure 5). As a consequence,
CD127 expression on central memory and effector memory CD4+ T cell subsets normalized after 24 months of HAART, although it still remained significantly reduced in effector and naive subsets.

CD38 expression declined with HAART in both CD4+ and CD8+ T cells. In CD4+ T lymphocytes, this decline was more noticeable in central memory and effector memory CD127− subsets; it was already significant by month 12 of HAART and continued thereafter. The same trend was observed in central memory and effector memory CD127+ subsets but with a significant decline only at month 24 of treatment. There was no significant change in CD38 expression on naive and effector subsets. At month 24, activation levels were similar to those observed in HIV-seronegative control subjects for most CD4+ T cell subsets, except for effector memory and effector subsets not expressing CD127, in which CD38 expression still remained significantly increased (figure 6). Activation levels significantly declined during HAART in all CD8+ T cell subsets (both CD127+ and CD127−), except CD127− central memory cells, in which activation remained at very high levels during the 24 months of successful HAART. Interestingly, they were similar to those seen in HIV-seronegative control subjects.

Factors influencing increases in CD4+ cell population with HAART. Linear regression analyses were performed to ascertain which baseline variables were associated with the extent of the increase in CD4+ cell population after 24 months of complete HIV suppression during HAART. For each patient, the change in CD4+ cell count at month 24 was expressed as a percentage of the baseline value. In the bivariate analysis, several baseline parameters were significantly associated with an in-
increase in the CD4+ cell population, including CD4+ cell count, serum IL-7 level, and CD127 expression and activation on different CD4+ and CD8+ T cell subsets (data not shown). In the multivariate analysis, only 3 baseline parameters were significantly and independently associated with an increase CD4+ cell population at month 24 of HAART: CD127 expression on central memory CD8+ T cells, CD4+ cell count, and IL-7 level. The variable that most contributed to the model was CD127 expression, followed to a much lesser extent by CD4+ cell count and finally by IL-7 level (table 1). According to this model, patients with lower baseline CD4+ cell counts, lower CD127 expression, and higher IL-7 levels had the greatest increase in CD4+ cell population after 24 months of successful HAART.

**DISCUSSION**

Factors that influence CD4+ cell depletion over the course of HIV infection and CD4+ cell restoration after suppression of viral replication with HAART are not well understood [14]. Although some of them, such as immune activation, are well established as significant contributors [15, 16], the roles of the others remain unclear. Recently, the IL-7–CD127 system has been shown to be involved in the pathogenesis of HIV immunodeficiency [24, 25]. To further investigate its role, we measured serum IL-7 level and the expression of CD127 in several CD4+ and CD8+ T cell subsets. The level of immune activation was examined in parallel, and all subjects were followed up for 24 months after complete viral suppression with HAART. A group of age-matched, HIV-seronegative individuals was used as control subjects.

Serum IL-7 level was increased and CD127 expression on CD8+ T cells was decreased in untreated patients with HIV infection, in agreement with the results observed in other reports [20, 24, 25]. The greatest down-regulation of CD127 was seen in effector and effector memory CD8+ T cells. A recent study that used different markers to define the maturation stage of T cells has also found the highest reduction of CD127 in effector CD8+ T cells [24]. Because the positive effects of IL-7 on the survival of T cells are mediated through interaction with CD127, cells lacking this marker might show a diminished proliferative potential [24]. Moreover, high levels of serum IL-7, as we saw in untreated patients, might induce an up-regulation of Fas on T cells, increasing their susceptibility to apoptosis [26]. Although CD127 down-regulation was also seen among CD4+ T lymphocytes, it was particularly pronounced for CD8+ T cells, in agreement with the findings of another study that showed a massive expansion of CD8+ T cells with effector phenotype not expressing CD127, including cells specific for HIV [23]. These cells showed a limited proliferative potential and increased rate of apoptosis. A role for the HIV Tat protein has been postulated for CD127 down-regulation in CD8+ T cells [27].

Down-regulation of CD127 was not limited to effector and effector memory CD8+ T cells. It was also noticed in central memory cells, a population more dependent on CD127 for long-term maintenance of immunological memory [28]. The population of long-lived functionally competent CD8+ memory T cells specific for HIV is preserved only in patients receiving treatment early after acquiring HIV infection [29]. CD127 was also down-regulated in naïve CD8+ T cells. In fact, this was the subset

**Table 1. Variables associated with increase in CD4+ cell population at month 24 of successful highly active antiretroviral therapy (HAART).**

<table>
<thead>
<tr>
<th>Variables included in the regression modela</th>
<th>Variation in CD4+ cell increase explained by the model, %</th>
<th>Regression coefficient (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation in CD45RA–CD27 b cell count</td>
<td>46</td>
<td>−0.013 (0.003)b</td>
<td>.001</td>
</tr>
<tr>
<td>CD4+ cell count</td>
<td>13</td>
<td>−0.0001 (0)c</td>
<td>.007</td>
</tr>
<tr>
<td>Plasma interleukin-7 level</td>
<td>7</td>
<td>0.46 (0.18)d</td>
<td>.02</td>
</tr>
</tbody>
</table>

*NOTE.* Increase in CD4+ cell population was expressed as the logarithm of the percentage of the baseline value. The analysis was multivariate.

a For the variables analyzed, CD127 expression was expressed as a percentage, CD4+ cell counts in cells per microliter, and interleukin-7 level in log units.

b Variation in CD4+ cell count (logarithm of percentage units) at month 24 of HAART for each incremental unit in baseline CD8+/CD45RA–CD27–CD127– cell count.

c Variation in CD4+ cell count (logarithm of percentage units) at month 24 of HAART for each incremental unit in baseline CD4+ cell count.

d Variation in CD4+ cell count (logarithm of percentage units) at month 24 of HAART for each log incremental unit of baseline plasma interleukin-7 level.
most affected, and the difference in CD127 expression between patients and control subjects was the highest for this cell population. Naive cells are initially CD127hi and down-regulate this marker after successive divisions and maturation toward effector cells [30]. In this scenario, the low levels of CD127 we saw in naive CD8+ T cells might reflect the increased T cell turnover and division characteristic of HIV infection. Changes in CD127 expression were less pronounced in CD4+ T cells, suggesting a different homeostatic mechanism for CD4+ and CD8+ T lymphocytes in HIV infection. A significant decrease in CD127 expression was seen only in effector memory CD4+ T cells. Because this subset expands in HIV-infected patients, down-regulation of CD127 might affect the regenerative potential of CD4+ T cells in the periphery, contributing to the gradual loss of the CD4+ cell pool.

An inverse association between CD127 expression on T cells and serum IL-7 level in vivo has already been reported [22, 24]. In vitro studies have shown that culture of PBMCs in the presence of IL-7 results in down-regulation of CD127, which is rapidly reversible on removal of IL-7 [22]. More recently, this effect has been shown specifically in CD8+ T cells [31]. Assuming that the in vitro results can be extrapolated to the in vivo situation, the high level of serum IL-7 seen in most HIV-infected patients should be the cause rather than the effect of CD127 down-regulation. However, data from long-term nonprogressors contradict this hypothesis, because these individuals show low CD127 expression despite having a normal serum IL-7 level [22]. Interestingly, the multivariate analysis we performed showed that CD127 down-regulation did not correlate with serum IL-7 levels but did correlate with cellular activation. To our knowledge, this is the first evidence of such association after adjustment for other factors, such as IL-7 level, CD4+ cell count, and plasma HIV RNA level.

The association between CD127 expression and T cell activation was negative, meaning that individuals who showed the highest activation displayed the least CD127 expression. In accordance with that result, T cells lacking CD127 expressed the highest amount of CD38. Moreover, the highest CD38 level in HIV-infected patients was seen in effector memory and effector T cell subsets that were indeed the most affected by CD127 down-regulation. These results suggest that T cell activation and CD127 down-regulation are tightly connected in HIV infection. The only exception could be naive CD8+ T cells, in which CD127 was highly down-regulated despite lack of activation. Other HIV-associated factors, such as the increased proliferation and/or direct effects of HIV proteins, might induce CD127 down-regulation in this cell population [27, 30].

The evolution of IL-7–CD127 and their influence on increases in the CD4+ T cell population was assessed during successful control of HIV replication with HAART. Another cross-sectional study found a significant increase of CD127 expression in T cells of patients undergoing long-term antiretroviral therapy, although normalization was not complete [25]. We extended this observation by longitudinally assessing CD127 expression in different T cell subsets. A significant increase of CD127 expression in all but naive CD8+ T cell subsets was seen. After 24 months of HAART, CD127 expression normalized in memory CD8+ T cells but still remained low in naive and effector subsets. CD127 expression on CD4+ T cells was less affected by HAART and still remained low in effector memory cells after 24 months of therapy. Overall, the increase in CD127 expression paralleled a decrease in T cell activation. However, activation was normalized in most CD8+ T cell subsets, including naive cells and effector cells in which CD127 did not normalize. The lack of association between CD127 expression on naive CD8+ T cells and immune activation is in agreement with this finding. In the case of effector cells, different kinetics in the 2 parameters may explain these results. Alternatively, effector CD8+ cells that remain CD127- in the setting of normal activation may include cells specific for HIV and other viruses, such as Epstein-Barr virus or cytomegalovirus [32].

After successful control of HIV replication with HAART, the CD4+ T cell population increased in all but 1 patient. Baseline factors potentially associated with an increase in the CD4+ cell population were examined. Serum IL-7 level has been positively correlated with CD4+ cell restoration after HAART [21]; however, this observation has not been confirmed by others [22]. In our multivariate analysis, a significant and positive correlation was found between serum IL-7 level and increase in CD4+ cell population after 24 months of HAART. Shorter follow-up periods in other studies could explain discrepancies [22]. It has to be pointed out, however, that in our multivariate model the main determinant of CD4+ cell restoration was CD127 expression on central memory CD8+ T cells, with IL-7 contributing only marginally to the model. Surprisingly, the association with CD127 expression was negative, meaning that greater increases in CD4+ cell population were seen in patients with lower baseline CD127 expression. After HAART, these patients are the ones experiencing more pronounced increases in CD127 expression. Thus, increases in CD4+ cell population seem to be highly dependent on the ability to normalize CD127 expression. In untreated patients with HIV infection with nearly normal levels of CD127 expression despite increased serum IL-7, the IL-7–CD127 system might be impaired. This hypothesis fits with the results of an in vitro study that showed a dysfunction of this system in both CD4+ and CD8+ T lymphocytes [33], and it also fits with the loss of correlation between CD127 expression and responsiveness to IL-7 in T cells from HIV-infected patients [25].

In summary, down-regulation of the IL-7 receptor is mainly associated with T cell activation and is only partially reverted after complete suppression of detectable plasma HIV RNA with HAART. CD127 expression on CD8+ T cells is the main determinant of the extent of increases in CD4+ cell population during receipt of HAART. Taken together, these results support an im-
important role for the IL-7–CD127 system in the pathogenesis of CD4+ cell depletion in HIV-infected patients. By contrast, they do not support the use of IL-7 as an adjuvant to boost immune reconstitution in these patients, and therefore other strategies aimed at the functional restoration of the IL-7–CD127 system must be sought.

References