Rapid Effect of Interleukin-2 Therapy in Human Immunodeficiency Virus–Infected Patients whose CD4 Cell Counts Increase Only Slightly in Response to Combined Antiretroviral Treatment

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Combined antiretroviral treatment in some human immunodeficiency virus–infected persons does not lead to a rapid increase in CD4 cell counts, and these patients may remain susceptible to opportunistic infections. A group of 13 patients with CD4 cell counts <200 cells/mm² after ≥9 months of combined antiretroviral treatment received interleukin (IL)–2 immunotherapy (4.5 × 10⁶ IU twice daily for 5 days every 6 weeks). After only 3 cycles, their CD4 cell counts increased from 123 cells/mm² (range, 104–134 cells/mm²) to 229 cells/mm² (range, 176–244 cells/mm²). A marked increase was noted in the naive CD45RA subpopulation of CD4 T lymphocytes. Furthermore, the magnitude of the CD4 cell count response correlated with the baseline expression levels of the antiapoptotic molecule Bcl-2. This study demonstrates that IL-2 immunotherapy can accelerate the recovery of CD4 lymphocytes in persons whose CD4 cell counts fail to increase rapidly in response to combined antiretroviral treatment.

In the treatment of human immunodeficiency virus (HIV)–infected patients, combined antiretroviral treatment regimens that usually include 2 reverse-transcriptase inhibitors (RTIs) and 1 protease inhibitor (PI)—called triple combination therapy or highly active antiretroviral therapy (HAART)—result in increased CD4 cell counts in conjunction with reduced plasma virus load. These patients show improved survival [1–3]; however, some patients have complete treatment failure and others show a discrepant response to these regimens [4–6]. Some have increased CD4 cell counts but with minimal virologic benefit, whereas others, here called CD4 low-responder (CD4-LR) patients, fail to exhibit a significant increase in CD4 cell counts, despite a marked reduction in virus load. CD4 cell counts increase only slowly in CD4-LR patients who continue to have CD4 cell counts <200 cells/mm² for several months. Although CD4-LR patients benefit from HAART, they may remain susceptible to opportunistic infections related to HIV infection [5, 7]. The proportion of CD4-LR patients is 5%–27% of those receiving HAART [4–6]. The reversibility of this therapeutic limitation has not yet been studied.

Interleukin (IL)–2, a CD4 T lymphocyte–produced cytokine, has a number of potent effects on the immune system. It promotes the proliferation and modulates the secretory capacities of T, B, and NK cells [8, 9]. Studies initially evaluated the effects of intravenous infusions of IL-2 in combination with antiretroviral drugs for the treatment of HIV infection [10, 11]. More recently, subcutaneous IL-2 cycles (1 week of IL-2 administration followed by a few weeks without treatment) has been efficient in increasing CD4 cell counts and in reducing side effects. Most IL-2 clinical trials have considered patients with moderate to high CD4 cell counts [11–19], but the effects of IL-2 in CD4-LR patients have not been investigated.

Recombinant IL-2 may be particularly useful in the treatment of CD4-LR patients, in whom it could induce a significant increase in circulating CD4 T lymphocytes. This increase in CD4 cell counts could reduce the risk of opportunistic infections, which are the hallmark of AIDS. We therefore designed a pilot study to explore the capacity of IL-2 to rapidly increase CD4 cell counts. When this trial was started, no reports had been published concerning immunotherapy in CD4-LR patients. The main goal of the present pilot study was to determine the effects of 3 IL-2 cycles (4.5 × 10⁶ IU twice daily for 5 days every 6 weeks) as a precursor to a large clinical end-point study of IL-2 treatment in CD4-LR patients.

Methods

IL-2 treatment. IL-2 (Aldesleukin; Chiron-Europe) was administered twice daily as a single subcutaneous injection of 4.5 MIU for 5 consecutive days (D1–D5). One cycle was defined as IL-2 administration for 5 days with concomitant antiretroviral treatment, followed by 5 weeks of antiretroviral treatment alone. The dose (9 MIU) and the frequency (6 weeks) were chosen to obtain a rapid response with limited toxic effects, on the basis of the results of other trials [11, 12, 14–17]. The antiretroviral treat-
ment remained the same throughout the IL-2 cycles. Day 1 cycle 1 (D1C1) for all patients corresponded to the first day of the first IL-2 cycle and D1C4 to the last day of the third cycle (or the first day of the fourth cycle).

All patients were required to complete a minimum of 3 IL-2 cycles and were evaluated at D1C4. The first IL-2 cycle was administered on an inpatient basis and the rest on an outpatient basis, except for persons who requested inpatient treatment for personal reasons. The patients were evaluated clinically on days 1, 5, 14, and 28 of the first cycle and on days 1 and 21 of the following cycles. Several biologic parameters, including CD4 cell counts, were checked at all time points by standard laboratory methods. Laboratory and clinical side effects were graded according to World Health Organization criteria.

**Flow cytometric analysis of CD45 isoforms and of Bcl-2 expression.** The expression of CD45RA and CD45RO on the cell surface of CD4 T lymphocytes was measured as described elsewhere [20] by using anti-CD45RA (UCHL1, IgG2a) monoclonal antibody (MAb; Dako) and anti-CD45RA (2H4, IgG1) MAb (Immunotech). Other MAbs, isotype-matched controls, and the Fab fragment used were identical to those used in our previous studies [20]. Results were expressed as percentage of CD4 T lymphocytes that expressed the molecule being considered.

Intracellular Bcl-2 was detected as follows: after staining of the CD4 cell surface [20], the cells were fixed in 1% paraformaldehyde in PBS, then were washed and permeabilized with a solution of 0.05% (wt/vol) saponin detergent in PBS. The cells then were incubated with fluorescein isothiocyanate–conjugated anti–Bcl-2 MAb (129, IgG1; Dako), and results were expressed as mean fluorescence intensity, because most cells expressed this molecule at different concentrations.

**Statistical analysis.** Statistical analyses were done with StatView 5 statistical software (Abacus Concepts). We used the nonparametric Mann-Whitney U test to compare nonpaired values (patients in immediate arm [IA] vs. patients in delayed arm [DA], see below). The nonparametric Wilcoxon paired rank test was used to analyze paired data (intrapatient baseline and follow-up) during HAART plus IL-2 treatment. We used the nonparametric Spearman test to analyze correlation. Results are shown as the median and range of quartiles (in parentheses).

**Results**

**CD4-LR patients.** HIV-positive patients were eligible if they had been receiving stable antiretroviral treatment for ≥9 months before study entry with resulting CD4 cell counts <200 cells/mm³ and a virus load <50 copies/mL. Patients were excluded if they were receiving corticosteroids or chemotherapy or showed abnormal liver, kidney, or thyroid function or experienced severe opportunistic infections. In all, 13 CD4-LR patients were recruited between May and July 1998 from a cohort of patients followed at the Hôpital de l’Institut Pasteur (Paris). Baseline characteristics are summarized in table 1. The HAART regimen was 2 RTIs plus 1 PI (n = 10) or 2 RTIs plus 2 PIs (n = 3). All drugs were given at standard doses. A substantial decrease in plasma virus load was noted in these patients during HAART (table 1). Over the same period, their CD4 cell counts had increased by only 37 cells/mm³ (range, 18–52 cells/mm³; table 1). No general or significant pattern in CD4 cell count variations was detected.

**Specific and rapid increase in CD4 cell counts in CD4-LR patients given IL-2.** One week after inclusion, the patients were randomized to receive IL-2 immediately (IA) or 12 weeks later (DA; figure 1A). During the first 12 weeks, IA patients (n = 7, 1 with PI) received IL-2 treatment in addition to the unchanged HAART. The first IL-2 cycle began at week 0 and the second at week 6. CD4 cell counts had increased substantially in the IA group at the end of the 12 weeks: 32 cells/mm³ (range, 28–39 cells/mm³). DA patients (n = 6, 2 with PI) received only HAART during these 12 weeks, and their CD4 cell counts increased only marginally: 6 cells/mm³ (range, 0.5–12 cells/mm³). The DA patients therefore corresponded to a control group for the IA patient group during the first 12 weeks of the trial. The difference at week 12 between the IA and DA patients with regard to increased CD4 cell counts was significant (P < .04). The DA patients then began the IL-2 treatment. Their CD4 cell counts also increased after 2 IL-2 cycles: 40 cells/mm³ (range, 33–65 cells/mm³). The variations in both arms show that IL-2 triggers increases in CD4 cell counts.

IL-2 treatment triggered an increase in CD4 cell counts from D1C1 to D1C4 in all patients (figure 1B) from 123 cells/mm³ (range, 104–134 cells/mm³) at baseline (D1C1) to 229 cells/mm³ (range, 176–244 cells/mm³) at D1C4, an increase of 86% (P < .006). The CD4 cell counts were analyzed for all 13 patients at the 4 points illustrated in figure 1B. The CD4 cell count increase followed the same pattern in each patient. Furthermore, the

<table>
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* Median (quartile range).
Figure 1. Increases in CD4 cell counts during interleukin (IL)-2 immunotherapy.

A. Median and quartile increases in CD4 cell counts (vs. baseline) in immediate arm (IA) and delayed arm (DA). IA patients (n = 7) received first 2 IL-2 cycles at weeks 0 and 6. DA patients (n = 6) received first 2 IL-2 cycles at weeks 12 and 18. P < .04 for differences in CD4 cell count increases at week 12 between IA and DA patients. 

B. Median and quartiles of CD4 cell counts during first 3 IL-2 cycles for all patients. CD4 cell counts are shown at baseline (day 1, cycle 1 [D1C1]), at midcycle (D14 or D21), and at end of each IL-2 cycle (D1C2, D1C3, and D1C4). Increase in CD4 cell counts between D1C1 and D1C4 is significant (P < .006). 

C. Increases in CD4 cell counts with highly active antiretroviral therapy (HAART) alone and with HAART plus IL-2 for all patients. Line shows increases in CD4 cell counts with HAART alone (duration, 19 ± 1.8 months) and increases in CD4 cell counts with HAART plus IL-2 (D1C1–D1C4: 18 weeks).

Biologic evaluation at midcycle (D14 for cycle 1, D21 for the others) showed a more substantial increase than that observed at the end of the cycles (figure 1B). Thus, CD4 cell counts increased rapidly after the IL-2 injections then partially decreased during the second half of the cycle. The overall result was a progressive increase in CD4 cell counts (figure 1B).

The increases noted in CD4 cell counts during HAART alone and during HAART plus IL-2 were compared for all patients (figure 1C). When combined antiretroviral treatment was given alone, the median increase in CD4 cell counts was ~2 cells/mm$^3$ per month; this increased to 25 cells/mm$^3$ per month with IL-2 treatment, further suggesting that the increase in CD4 cell counts was due to the IL-2 injections.

Characterization of increases in CD4 T lymphocytes. Figure 2 shows a progressive increase in the percentage of CD4$^+$ lymphocytes (figure 2A) and in the CD4:CD8 ratio (figure 2B) during IL-2 treatment in CD4-LR patients. CD4$^+$ lymphocytes increased by 47% after 3 IL-2 cycles, and the CD4:CD8 ratio increased by 106%. These variations were significant (P < .03 and P < .01, respectively).

Because the origin of the CD4 T lymphocytes produced during IL-2 immunotherapy is not completely understood, we measured CD45RO (i.e., memory) cells and CD45RA (i.e., naive) cells at D1C1 and D1C4 (figure 2C) and found a significant increase in CD45RA-positive cells (from 29% to 42%; P < .03). As a result, the RA:RO ratio increased significantly in all patients (82%). In addition, we found a negative correlation between the percentage of CD4 cell T lymphocytes positive for CD45RA at baseline and the relative variation in their percentage from D1C1 to D1C4 (figure 2D). The same negative correlation was found with the percentage increase in CD4 cell counts. This observation does not support the hypothesis of a peripheral expansion of CD45RA naive cells during IL-2 immunotherapy.

Effect of baseline Bcl-2 expression on increase in CD4 cell counts during IL-2 treatment. Lymphocyte apoptosis is an important phenomenon in HIV infection [21, 22], and HAART increases CD4 cell counts by various mechanisms, including an effect on apoptosis [23]. Because we found no expansion of CD4 T lymphocytes in CD4-LR patients, we hypothesized that CD4 T lymphocytes in these patients were particularly susceptible to apoptosis. We therefore measured the expression of Bcl-2, an important molecule for protecting cells from apoptosis. Bcl-2 expression is diminished during HIV infection, and combined antiretroviral therapy can restore this expression and limit apoptosis [24, 25]. Figure 3A shows that baseline Bcl-2 (D1C1) is underexpressed in the CD4 T lymphocytes of CD4-LR patients, compared with the expression in HIV-negative healthy persons. We analyzed the effect of the heterogeneity of baseline Bcl-2 expression on the magnitude of the CD4 cell count increase after IL-2 therapy. Figure 3B (left panel) shows Bcl-2 expression for each patient at baseline and the percentage increase in CD4 cell counts during the first 3 IL-2 cycles. By statistical analysis, these 2 parameters had a significant positive correlation. In contrast, we were unable to establish any correlation between the CD4 cell count increase and CD4 cell counts at D1C1, HAART duration, or CD4 cell count variation during HAART (data not shown). In the 7 patients studied at D1C6, we found a correlation between the percentage increase in CD4 cell counts and baseline Bcl-2 expression (figure 3B, right panel).
IL-2 in HIV-Positive Patients with Low CD4 Cell Counts

Figure 2. Increases in percentage of CD4+ lymphocytes, CD4:CD8 ratio, and CD45RA:CD45RO ratio during interleukin (IL)-2 immunotherapy. A, Medians and quartiles of CD4+ lymphocytes at baseline and at ends of first 3 IL-2 cycles. Increase in CD4-positive lymphocytes between day 1, cycle 1 (D1C1) and D1C4 is significant (P < .03). B, Medians and quartiles of CD4:CD8 ratio at baseline and at end of each IL-2 cycle. Increase in CD4:CD8 ratio between D1C1 and D1C4 is significant (P < .01). C, Medians and quartiles of CD45RA:CD45RO ratio at D1C1 and D1C4; increase in CD45RA:CD45RO ratio from D1C1–D1C4 is significant (P < .03). D, Each dot corresponds to percentage of CD4 T lymphocytes positive for CD45RA at baseline (X-axis) and relative variation from D1C1–D1C4 (Y-axis) per patient. Relative variation in CD4 T lymphocytes positive for CD45RA is calculated as follows: (percentage of CD4 T lymphocytes positive for CD45RA at D1C4 – percentage of CD4 T lymphocytes positive for CD45RA at D1C1)/percentage of CD4 T lymphocytes positive for CD45RA at D1C1. Negative correlation between these 2 values is significant (r = −.86; P < .007, Spearman test).

Discussion

Current standard therapy for HIV infection (combined antiretroviral regimens including PIs) protects the immune system from damage by inhibiting viral replication and thus delays the onset of opportunistic infection and prolongs survival [1, 2]. However, CD4-LR patients do not show any rapid reconstitution of the CD4 T lymphocyte pool, despite efficient blockage of viral replication, and may remain susceptible to opportunistic infections [5, 7]. Thus, we considered it important to treat these patients with IL-2, to evaluate their ability to increase CD4 cell counts and to promote reconstitution of their immune systems.

The IL-2 regimen used consisted of successive IL-2 cycles (4.5 × 10^6 IU twice daily for 5 days). This treatment was given subcutaneously every 6 weeks. The IL-2 injections were well tolerated by all patients and did not hinder daily lifestyle in most patients. Thus, it appears that IL-2 can be given to CD4-LR patients without inducing new or unusually severe side effects, compared with results of other trials involving patients in a less critical clinical condition [11, 12, 14–17]. Plasma virus load did not vary significantly during the IL-2 treatment period. Although median cellular virus load at D1C4 was lower than at D1C1, this decrease did not reach statistical significance. Furthermore, no opportunistic infections occurred in our patients during this trial or in the following months.

CD4-LR patients were selected to receive IL-2 immunotherapy if, after 9 months of combined antiretroviral treatment, they had CD4 cell counts <200 cells/mm^3. Above this threshold level, patients are potentially protected against opportunistic infections, and some prophylactic treatments are discontinued [26–30]. Our study shows that IL-2 immunotherapy can increase CD4 cell counts in CD4-LR patients. In these patients, the benefit of IL-2 treatment is 2-fold: first, CD4 cell counts are significantly increased, compared with those of persons...
given combined antiretroviral treatment alone, and second, CD4 cell counts increase rapidly. After only 3 cycles, CD4 cell counts increased by >80%. We measured all other hematologic lineages, in addition to the CD4 T lymphocytes, and none varied during IL-2 immunotherapy.

In view of the success obtained with the short-term treatment protocol in CD4-LR patients, they were given additional IL-2 cycles, and their CD4 cell counts continued to increase progressively. At the end of IL-2 cycle 3, 11 of 13 patients had CD4 cell counts >200 cells/mm³. Seven patients received 9 IL-2 cycles and had a median CD4 cell count of ~500 CD4 cells/mm³, an increase of >300%. During these additional IL-2 cycles, we again noted only sporadic, moderate plasma virus loads and no change in the frequency or the intensity of side effects. When IL-2 was discontinued, the CD4 cell counts appeared to remain stable. When we considered the mechanisms by which CD4 cell counts increased, our data indicated that IL-2 immunotherapy resulted in the preferential appearance of naïve (CD45RA-positive) lymphocytes (figure 2C), as observed elsewhere [11, 15, 17]. The origin of the naïve cells in both combined antiretroviral therapy and in IL-2 immunotherapy is not well understood. Several origins have been explored in combined antiretroviral therapy (e.g., neothymic output, peripheral expansion, and reversion from CD45RO to CD45RA) [31–35]. Here, we found a negative correlation between the percentage of naïve cells at baseline and their relative increase during IL-2 immunotherapy. This finding does not support the hypothesis that peripheral expansion is the unique origin of the naïve population, but further investigations are needed to establish firm conclusions [36, 37].

The CD4 cell counts observed after IL-2 immunotherapy were heterogeneous and did not appear to be related to baseline CD4 cell counts. Our analysis furnishes a possible explanation for this heterogeneous response, because we saw a link between baseline Bcl-2 expression and the percentage increase in CD4 cell counts at D1C4. Bcl-2 is an intracellular protein that protects cells from apoptosis triggered by a withdrawal of growth factors [38]. Furthermore, Bcl-2 can control the apoptosis of lymphocytes in HIV-infected persons [39]. Thus, it may be hypothesized that the pool of IL-2–responding cells is controlled by Bcl-2 and that IL-2 immunotherapy would be more effective in persons with a high proportion of nonapoptotic cells (i.e., with high Bcl-2 levels). Furthermore, because IL-2 treatment induces Bcl-2 expression (data not shown), it would reduce the number of apoptotic cells and further increase the pool of responding cells. The use of baseline Bcl-2 expression to predict the subsequent increase in CD4 cell counts should be further explored in a larger number of patients. It would also be of interest to determine whether this correlation can also be extended to other clinical situations.

In conclusion, we believe that the present study is the first to report that IL-2 immunotherapy can accelerate the recovery of CD4 cell counts in CD4-LR patients. The rapid increase in naïve CD4 T lymphocytes (CD45RA) in these patients may be critical. The potential of these cells to protect against opportunistic infections remains to be established. However, CD4 cell counts rapidly exceeded the threshold considered to be protective. Furthermore, we established that baseline Bcl-2 levels may be predictive of the subsequent CD4 cell count response to IL-2 immunotherapy. This study therefore provides a solid basis in favor of large clinical trials aimed at defining the effects of IL-2 in the treatment of HIV-infected patients with low CD4 cell counts.

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References

12. Kovacs JA, Vogel S, Albert JM, et al. Controlled trial of interleukin-2 in...