Efficacy of Low-Dose Subcutaneous Interleukin-2 to Treat Advanced Human Immunodeficiency Virus Type 1 in Persons with \(\leq 250/\mu L\) CD4 T Cells and Undetectable Plasma Virus Load

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The immunologic efficacy of low-dose recombinant interleukin-2 (rIL-2) administered subcutaneously (sc) once a day in combination with highly active antiretroviral therapy (HAART) was assessed in a pilot study in patients with advanced human immunodeficiency virus (HIV) disease. Twenty-five persons with \(\leq 250\) CD4 cells/\(\mu L\) and plasma HIV-1 RNA levels \(\leq 500\) copies/mL for \(>24\) weeks were randomly assigned to receive sc rIL-2 (\(3 \times 10^6\) IU once a day) with their previous antiretroviral regimen (\(n = 13\)) or to continue with the same treatment (\(n = 12\)). The level of CD4 T cells was significantly higher in the IL-2 group at week 24 (105 ± 65/\(\mu L\); \(P < .05\)) but not in the control group (30 ± 78/\(\mu L\)). Memory T cells initially contributed to the CD4 T cell increase at week 4 (\(P < .05\)). Naive T cell increases (99 ± 58/\(\mu L\)) in the IL-2 group became statistically significant at week 24 compared with the control group (28 ± 27/\(\mu L\); \(P < .05\)). Subcutaneous rIL-2 once a day in combination with HAART was well tolerated and improved immunologic surface markers in patients with advanced HIV infection.

In some persons infected with human immunodeficiency virus (HIV) type 1, highly active antiretroviral therapy (HAART) produces significant immunologic improvements with increases in memory and naive T lymphocytes [1]. However, in others, CD4 T cells do not significantly increase, despite a substantial decrease in plasma virus load. It has been suggested that the development of immune-based therapeutic strategies [2, 3] might be useful for attaining long-term control of HIV infection.

Interleukin (IL)-2 is a cytokine produced by T cells that promotes proliferation and modulates the secretory capacity of all major types of lymphocytes, including T, B, and natural killer cells [4, 5]. Specificity of the immune response depends on the antigen triggering the entry of T cells in the cell cycle (G0–G1), but the proliferation of antigen-activated cells is dependent on IL-2, which induces the beginning of the S phase (G1–S), a crucial step for cell proliferation [6].

Several studies have evaluated the effects of intermittent continuous intravenous infusions of IL-2 in combination with antiretroviral drugs [7, 8]. Most of those trials focused on patients with \(>200\) CD4 T cells/\(\mu L\) and demonstrated substantial and sustained increases in CD4 T cells, with transient or no associated increase in plasma HIV-1 RNA levels [7, 8]. However, there are few reported studies in which IL-2 has been administered to patients with advanced HIV infection [7, 9].

Recombinant (r) IL-2 may be particularly useful in patients with advanced HIV disease, in whom it could induce a significant increase in the number of circulating CD4 cells. An increase in CD4 T cells would reduce the risk of opportunistic diseases that are the hallmark of AIDS [10–12]. Nevertheless, the potential risk of virus load rebound in persons with advanced HIV-1 disease who take IL-2 has not been fully investigated. Therefore, we conducted a pilot study to assess the immunologic efficacy and tolerability of intermittent low doses of subcutaneous (sc) rIL-2 in combination with HAART in persons with advanced HIV-1 disease.

**Patients and Methods**

**Study population.** Patients \(\geq 18\) years who were seropositive for HIV-1 and had \(\leq 250\) CD4 T cells/\(\mu L\) and plasma HIV-1 RNA levels \(\leq 500\) copies/mL for \(>24\) weeks prior to randomization were eligible for this study. Patients had to be treated with protease inhibitor (PI)-containing regimens for \(\geq 6\) months before their re-
recruitment. In addition, the screened patients included in the study were nucleoside analogue–experienced before receiving triple therapy containing PIs. Patients were excluded if they had previous experience with IL-2 therapy or lacked stable antiretroviral therapy in the 6 months before study entry.

**Study design.** A randomized, open-label, controlled pilot study compared the efficacy of low-dose sc rIL-2 in combination with antiretroviral treatment versus antiretroviral treatment alone in HIV–advanced patients. The administered dosage of sc rIL-2 (Proleukin, Aldesleukin for injection; Chiron, Amsterdam, The Netherlands) was 3 × 10^6 IU twice a day for 5 days every 4 weeks for 6 cycles. The duration of the study was 24 weeks (6 cycles), although patients were followed until week 36. Patients were monitored throughout the study for safety and for immunologic and virologic changes every 4 weeks. Safety parameters included hemato logic, renal, and hepatic routine test.

**End point.** The primary end point of the study was to attain a significant increase in CD4 T cells in patients treated with IL-2 with respect to the control group while maintaining viral suppression <200 copies/mL in both groups.

**Sample size.** We hypothesized that an increase in CD4 cells of >60% could be attained in the IL-2 group. If we assumed an α error of 5% and a β error of 10%, the estimated number of patients required to achieve significant differences between groups was 12 subjects per arm.

**Flow cytometry and subpopulation analysis.** Two- or three-color fluorescence-activated cell flow cytometric analyses were performed on peripheral blood mononuclear cells (PBMC) isolated by ficoll-hypaque density gradient as previously described [13]. The lymphocyte subpopulations were determined at baseline and at weeks 4, 8, 12, and 24. Data from a minimum of 10^6 cells were collected for phenotype analysis. Live cells were electronically gated by the forward and side light scatter pattern. The direct immunofluorescence analysis included the following monoclonal antibodies (MAbs): X40 (negative control γ1 fluorescence isothiocyanate [FITC]), X39 (negative control γ2a-phycocyanin [PE]), SK1 (CD8-PE), SK3 (CD4-FITC), SK7 (CD3-biotin), 2D1 (CD45-FITC), M12P (CD14-PE), B73.1 (CD16-PE), MY31 (CD56-PE), L48 (CD45RA-FITC), UCHL-1 (CD45R0-PE), 2A3 (CD25-PE; Becton Dickinson, Mountain View, CA), and 37.51 (CD28-Biotin; Pharmingen, San Diego). For detecting biotin-labeled MAbs, we used FITC- or PercP-labeled streptavidin (Becton Dickinson) in a second step.

**Plasma virus load and proviral DNA measurements.** Plasma HIV-1 RNA levels were measured by a standard bDNA assay (Chiron, Madrid), which has a detection limit of 500 copies/mL, according to the manufacturer’s instructions [14]. Levels of HIV-1 RNA were quantified at screening, baseline, weeks 4, 8, 12, 16, 20, and 24, and at the end of each sc rIL-2 injection cycle (weeks 5, 9, 13, 17, and 21).

Quantification of HIV-1 proviral DNA in PBMC was performed at baseline and at week 24 by polymerase chain reaction amplification and limiting dilution format as described elsewhere [15]. Each dilution was run in duplicate. The lowest limit of detection was 1 proviral copy per 1 µg of DNA (10^6 cells). The accuracy was demonstrated by use of serial dilutions of LAV-8E5 cells, and the interassay variability was 0.22 log.

**Statistical analysis.** To evaluate the direct effect of rIL-2 in patients with ≤250 CD4 cells/mL, data were statistically analyzed as treated population. Surface cell markers at baseline and at weeks 4, 8, 12, and 24 were compared by Wilcoxon test. Differences between the IL-2–treated patients and the control group were assessed by the nonparametric Kruskal-Wallis test. All statistical tests were two-sided, and P ≤ .05 was considered statistically significant [16]. Data were analyzed by SPSS software (version 7.5; SPSS, Chicago).

**Results**

**Study sample.** Twenty-five of 256 patients fulfilling the entry criteria were consecutively selected from our database, which comprised 1576 HIV-1–infected subjects. These 25 patients were representative of the 256 individuals in terms of CD4 T cells and HIV-1 RNA levels. Thirteen patients were randomly assigned to the IL-2 group and 12 to the control group. Baseline characteristics were well balanced between groups (table 1). Likewise, at the time that HAART was initiated, IL-2 and control groups were comparable regarding CD4 cells (68 ± 43 and 73 ± 38/µL, respectively) and plasma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control groupa</th>
<th>IL-2 groupa</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>Mean 34</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Range 25-42</td>
<td>23-48</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Female 3</td>
<td>2</td>
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<tr>
<td>Risk factors</td>
<td>Heterosexual 5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Homosexual 6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Intravenous drug user 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hemophilic 1</td>
<td>1</td>
</tr>
<tr>
<td>CD4 cells/µL</td>
<td>Mean ± SD 129 ± 57</td>
<td>165 ± 77</td>
</tr>
<tr>
<td></td>
<td>Range 53-205</td>
<td>89-247</td>
</tr>
<tr>
<td>CD4 percentage</td>
<td>Mean ± SD 8 ± 6</td>
<td>12 ± 3</td>
</tr>
<tr>
<td></td>
<td>Range 2-26</td>
<td>7-17</td>
</tr>
<tr>
<td>CD8 cells/µL</td>
<td>Mean ± SD 986 ± 578</td>
<td>791 ± 379</td>
</tr>
<tr>
<td></td>
<td>Range 348-1809</td>
<td>222-1419</td>
</tr>
<tr>
<td>Log HIV proviral DNA/10^6 CD4</td>
<td>Mean 3.1 ± 0.7</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Range 2-3.6</td>
<td>2.1-3.8</td>
</tr>
<tr>
<td>Time on HAART before IL-2 therapy</td>
<td>Mean 11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Range 9-13</td>
<td>11-16</td>
</tr>
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**NOTE:** HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy; IL-2, interleukin-2; AZT, zidovudine; 3TC, lamivudine; IDV, indinavir; d4T, stavudine; RTV, ritonavir.

a Both groups maintained same antiretroviral treatment they were receiving before IL-2 initiation study.
virus load (4.9 ± 0.6 and 5.02 ± 1.1 log copies/mL, respectively). Progression, defined as the development of a new AIDS-defining event, was not observed in any participants during the study.

The IL-2 group received 3 × 10^6 IU twice a day of sc rIL-2 for 5 days during the first cycle. Because 80% of the subjects experienced high fever (>39°C) and severe systemic symptoms (grade 3 by the National Institute of Allergy and Infectious Diseases Division of AIDS table for grading adult adverse experiences), doses were reduced to 3 × 10^6 IU once daily in all subjects to improve tolerability and compliance.

Of 25 participants, 8 in the IL-2 group and 10 in the control group completed the study. Seventy percent of those receiving 3 × 10^6 IU per day of IL-2 had mild constitutional side effects (grade 2). The most common symptoms were fever, fatigue, and myalgias. Administration of antipyretic drugs (aspirin or paracetamol) 1 h after injection of rIL-2 controlled constitutional symptoms.

**CD4 T cell changes.** Peripheral absolute CD4 T cell counts increased significantly in the IL-2 group at week 24 compared with the mean baseline values (105 ± 65 μL; *P < .05), whereas in the control group the mean increase was not statistically significant (30 ± 78 μL). The mean increase in CD4 T cells was higher at the first rIL-2 cycle than in subsequent cycles (*P < .05; figure 1). The mean CD4 T cell count did not significantly change 3 months after the last IL-2 cycle.

Memory T cells (CD4^+CD45RO^+) initially contributed to the CD4 T cell increase at week 4 (*P < .05; figure 2). However, the subsequent progressive increase in CD4 T cells was consequent to the increase in naive cells (CD45RA^+), which began to be significant after the fourth cycle (week 12). The mean CD4^+CD45RA^+ T cell increase (99 ± 58 μL) in the IL-2 group became statistically significant at week 24 compared with the control group (28 ± 27 μL; *P < .05; figure 2).

The mean absolute and percent CD25 T cell marker increased significantly in the IL-2 group compared with the mean baseline value and with the control group (*P < .05; table 2). In the control group, this activation marker did not significantly change during follow-up.

We also analyzed the expression of CD28 on CD4 and CD8 T cells and found a relative increase in the number of CD4^+CD28^+ cells (156 ± 73 μL; *P < .05) at week 24 in the IL-2 group compared with baseline values (table 2). CD8^+CD28^+ cells increased from the beginning of the IL-2 therapy and reached a mean of 208 ± 270 μL at week 24. By contrast, in the control group the CD8^+CD28^+ cells decreased progressively over time (−303 μL; table 2).

**Plasma HIV-1 RNA and proviral DNA.** All patients except one maintained plasma HIV-1 RNA concentrations below the limit of detection (<500 copies/mL). This subject had a transient virus load increase after 1 IL-2 injection cycle (1570 copies/mL). HIV-1 proviral DNA decreased 6 months after initiation of HAART (data not shown). However, no significant proviral
DNA changes after IL-2 therapy were observed (from $3.3 \pm 0.5$ to $3.2 \pm 0.7 \log_{10}$ HIV proviral DNA copies/10$^9$ CD4 cells).

Patients withdrawn from the study. Overall, 7 patients were withdrawn from the study. Two patients were noncompliant (1 in the IL-2 group and 1 in the control group). Three patients changed antiretroviral treatment during the study because of intolerance to ritonavir (2 in the IL-2 group, 1 in the control group). Finally, 2 patients refused to continue the sc rIL-2 therapy after completing the first cycle. The 2 noncompliant subjects did not experience any significant change in CD4 cells from baseline. Plasma HIV-1 RNA concentration rose above 200 copies/mL (465 copies/mL) in the noncompliant patient in the control group. The 3 patients who changed antiretroviral therapy during the study had increased CD4 cell counts and maintained undetectable virus load. However, we could not determine whether this increase was induced by IL-2 therapy or by the new HAART combination. The 2 patients who refused to continue the sc rIL-2 therapy after the first IL-2 cycle had an increase in CD4 T cells (78 ± 58 and 106 ± 71/µL) 1 month after the onset of the study. This increase was maintained for 8 weeks and thereafter decreased to baseline values.

Discussion

Our study demonstrates the immunologic efficacy of intermittent low-dose sc rIL-2 ($3 \times 10^6$ IU once a day) in combination with HAART in HIV-1-infected persons with $\geq$250 CD4 T cells/µL. During the first cycle of sc rIL-2 therapy, we observed a significant increase in the CD4$^{45RO^-}$ subset, which decreased slightly afterwards. This initial increase in CD4$^{45RO^-}$ may be explained by the ability of rIL-2 to induce the transition from G1 to S in T cells [6]. In contrast, absolute numbers of CD4$^{45RA^-}$ did not increase significantly until the fourth cycle and then rose progressively. The initial CD4$^{45RO^-}$ T cell increases were similar to those in previous studies [1, 17] in which subjects were treated only with antiretroviral therapy. Moreover, as in the study by Connors et al. [18], we also saw CD4$^{45RA^+}$ T cell increases once IL-2 therapy was initiated.

It has been demonstrated that low doses of rIL-2 administered for a short period are sufficient to stimulate expression of the IL-2 receptor [19, 20]. Progressive increases in the number and percentage of CD25 T cells were observed in the IL-2 group, showing that our rIL-2 approach can stimulate the expression of the high-affinity receptor for IL-2.

Optimal stimulation and prevention of anergy in T cells require signaling through the CD28 molecule. During HIV disease progression, CD28 expression is progressively lost, particularly on CD8 T cells [21–23]. Our results show a proportional increase in the number of CD8$^+$CD28$^+$ cells in the IL-2 group, suggesting an improvement in costimulation through the second signal receptor and perhaps recovery in the CD8 cell function. Moreover, if we take into account that noncytotoxic response may be related to the fact that they had attained a reduction in proviral DNA, together with the short duration of our study, may have prevented the demonstration of an effect related to IL-2.

Clark et al. [24] observed a decrease in proviral DNA in persons treated with rIL-2 infusion plus zidovudine. In our patients, the absence of significant decreases in proviral DNA could be related to the fact that they had attained a reduction with HAART before the introduction of IL-2. Also, the slight slope of decrease in proviral DNA, together with the short duration of our study, may have prevented the demonstration of an effect related to IL-2.

The highest CD4 T cell increases, observed at week 4 with respect to the subsequent cycles, might be associated with the initial dose of sc rIL-2 ($3 \times 10^6$ IU twice a day). Nevertheless, the subsequent switch to $3 \times 10^6$ IU once a day seemed to be sufficient for sustaining the initial increase in CD4 T cells. In agreement with other studies [8, 9], we found that the immu-

<table>
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<tr>
<th>Surface marker</th>
<th>IL-2 group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>Absolute CD25 (cells/µL)</td>
<td>$161 \pm 69$</td>
<td>$225 \pm 175$</td>
</tr>
<tr>
<td>CD25 (%)</td>
<td>$12 \pm 3$</td>
<td>$15 \pm 7$</td>
</tr>
<tr>
<td>Absolute CD4 CD28 (cells/µL)</td>
<td>$141 \pm 54$</td>
<td>$161 \pm 80$</td>
</tr>
<tr>
<td>CD4 CD28 (%)</td>
<td>$12 \pm 5$</td>
<td>$17 \pm 9$</td>
</tr>
<tr>
<td>Absolute CD8 CD28 (cells/µL)</td>
<td>$681 \pm 384$</td>
<td>$936 \pm 544$</td>
</tr>
<tr>
<td>CD8 CD28 (%)</td>
<td>$56 \pm 12$</td>
<td>$45 \pm 14$</td>
</tr>
</tbody>
</table>

* By Mann-Whitney U test, difference between baseline and week 24 was not significant for all groups; difference between groups at end of study was <0.05 for all groups.
nologic effect produced by rIL-2 was prolonged for ≥3 months after the last cycle. Likewise, after the initial doses were reduced because of adverse effects, there was better tolerance of rIL-2 and sustained improvement in the different immunologic surface markers evaluated. We speculate that if doses of 3 × 10^6 IU twice a day or higher had been maintained, there would have been a greater effect on the immune system. Nevertheless, it is essential to balance tolerance with efficacy and to facilitate compliance by reducing daily administrations.

Whatever the mechanism involved in the improvement of the immunologic status [25–27], the use of immunomodulators concomitantly with HAART may shorten the time required to attain a reconstitution of the immune system in comparison with HAART alone. To our knowledge, this is the first to demonstrate the immunologic efficacy of sc rIL-2 in experienced and advanced patients receiving HAART, and it indicates that the combination of antiviral and immune-based therapy may further improve a deteriorated immune system. Further studies should be conducted, but our data are encouraging in this setting of advanced HIV disease.

Acknowledgments

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References


