Efficacy of recombinant interleukin-2 (rIL-2) in patients with advanced HIV-1 infection and blunted immune response to HAART

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OBJECTIVE. The efficacy of recombinant interleukin-2 (rIL-2) was assessed in HIV-infected patients with advanced immune suppression and a discordant immune response to highly active antiretroviral therapy (HAART). The primary endpoint was median change in CD4+ T-cell counts at the end of treatment as compared to baseline. Secondary endpoints were safety and changes in the various T-cell subpopulations.

MATERIAL AND METHODS. In a prospective cohort study, 19 patients with HIV-RNA < 50 copies/ml and < 200 CD4+ T cells/mm³ without a significant increase in the previous 12 months were scheduled to receive 6 cycles of 4.5 x 10⁶ IU subcutaneous rIL-2 daily for 5 consecutive days, every 4 weeks.

RESULTS. Median age was 43 years, and 64% had a previous AIDS-defining event. Median nadir and baseline CD4+ cell counts were 36 and 99 cells/mm³, respectively. Three patients discontinued treatment and one experienced grade 4 side effects. CD4+ T-cell counts increased to 147 cells/mm³ (range, 24-285) at 1 month following completion of treatment (P = 0.002), and 180 cells/mm³ (range, 38-280) at 18 months (P < 0.001). This improvement was associated with a significant decrease in expression rates of the activation markers, HLA-DR and CD38.

CONCLUSION. Our results suggest that in patients with advanced HIV infection showing a blunted immune response to HAART, rIL-2 might increase the pool of CD4+ T-cells by down-regulating the status of immune activation.

Key words: Recombinant IL-2. Discordant immune response. Regulatory CD4+ T cells.

Efficacia de la interleucina-2 recombinante (rIL-2) en pacientes con infección avanzada por VIH-1 y escasa respuesta inmunológica al TARGA

OBJETIVO. Evaluamos la eficacia de la interleucina-2 recombinante (rIL-2) en pacientes infectados por VIH, con inmunodepresión avanzada y respuesta inmune discordante al tratamiento antirretroviral de gran actividad (TARGA). El objetivo primario de evaluación fue el aumento de los linfocitos CD4+ al final del tratamiento respecto al valor basal. Se evaluó, además, la seguridad y el efecto de la rIL-2 sobre las diferentes poblaciones linfocitarias.

MATERIAL Y MÉTODOS. Estudio de cohorte prospectivo en el que se incluyeron 19 pacientes con ARN-VIH < 50 y linfocitos CD4+ < 200/mm³, estables en los 12 meses previos. Se administró rIL-2 a dosis de 4.5 x 10⁶ mUI/día por vía subcutánea, cada 4 semanas, hasta completar 6 ciclos.

RESULTADOS. La edad media de los pacientes fue 43 años, y 64% tenían un evento definitorio de AIDS. La mediana de los linfocitos CD4+ al inicio del estudio fue de 99 células/mm³, y 180 células/mm³ al final (P < 0.001). Este incremento se asoció con una disminución en la expresión de los marcadores de activación linfocitaria, HLA-DR y CD38.

CONCLUSIÓN. Estos resultados sugieren que la rIL-2, en pacientes con infección por VIH avanzada y respuesta inmune discordante al TARGA, puede contribuir a la expansión de los linfocitos CD4+ a través de una disminución de la activación inmunológica.

Palabras clave: IL-2 recombinante. Respuesta inmune discordante. Linfocitos CD4+ reguladores.

Introduction

A significant proportion of patients with advanced HIV-infection show incomplete immune reconstitution after initiation of highly active antiretroviral therapy (HAART) and may be at risk for opportunistic infections and clinical progression. Several trials have shown that...
Materials and methods

Study design

Nineteen Caucasian patients were enrolled in an open, prospective study from January 2001 to June 2003. Eligibility criteria included age 18 to 60 years, plasma HIV-RNA < 50 copies/mL stable HAART, CD4+ < 200 cells/mm³ with no significant increase in the last 12 months, and no AIDS-defining illnesses or steroid administration for at least one year. Patients were scheduled to receive 6 cycles of subcutaneous rIL-2 (Macrolin®), Chiron, France), consisting of 4.5 x 10⁶ IU daily for 5 consecutive days, every 4 weeks. Patients receiving at least one dose of rIL-2 were included in the safety analysis, based on changes in plasma HIV-RNA and laboratory parameters as well as clinical safety and tolerability. Blood samples for measuring plasma HIV-RNA, lymphocyte subset analysis, and T-cell production of intracellular cytokines were obtained on the first day of each cycle prior to dose administration, and one month after the last cycle. After completion of therapy, blood samples were obtained every three months. Efficacy was evaluated in patients receiving at least three cycles. The primary study endpoint was the median change in CD4+ lymphocyte counts at the end of treatment from baseline. Secondary endpoints were the percentage of patients who attained more than 200 CD4+ T-cells/mm³, changes in the percentage of lymphocyte phenotype subsets, assessment of intracellular IL-2, IFN-γ, IL-4 and TNF-α production, and the tolerance and safety of rIL-2 therapy.

Laboratory testing

Lymphocyte subpopulations were analyzed in lymph fresh whole blood samples by flow cytometry (FACS®Calibur; Becton Dickinson, San Jose, CA), and Cell-Quest software for acquisition and analysis. Intracellular production of IFN-γ, TNF-α, IL-4 and IL-2 was detected by flow cytometry after stimulating whole blood samples with PMA and ionomycin during 4 h in the presence of monensin, plus further lysis, permeabilization and cell staining with specific monoclonal antibodies. Non-stimulated whole blood was used as a negative control.

Statistical analyses

The data set was closed on May 31, 2005. A paired Wilcoxon signed-rank test was used to assess whether changes over time were different from zero. Correlations were studied with the non-parametric Spearman correlation coefficient. A two-sided P-value less than 0.05 was considered statistically significant. Multivariate analysis was not performed because of the small number of patients. Data analysis was performed with SPSS, v. 12.0 (SPSS Inc., Chicago, IL). All patients provided written informed consent for participation.

Results

Demographic and clinical characteristics of the patients are summarized in table 1.

Changes in CD4+ and CD8+ T lymphocytes

Overall, rIL-2 led to an increase in CD4+ T-cell counts from a median of 99 cells/mm³ (range, 36-185 cells/mm³) to 147 cells/mm³ (range, 24-285 cells/mm³) after the last dose of treatment (P = 0.002), and 180 cells/mm³ (range, 38-280 cells/mm³) 18 months after the last dose (P < 0.001) (table 2). The CD4+ T-cell percentage increased from 10% (range, 4-19) to 13% (range, 3-21) after the third cycle (P = 0.001) and remained stable afterwards (table 2). However, an individual-based analysis of CD4+ T-cell count change over time showed marked heterogeneity. Among 18 patients receiving at least 3 cycles of rIL-2, 5 (27.7%) experienced an increase in CD4+ cell counts lower than 25%, and 3 (17%) (P = 0.50) and 8 (44%) (P = 0.016) patients achieved >200 CD4+ lymphocytes/mm³ at the end of treatment and at month 18 post-treatment, respectively. Pre-treatment CD4+ T-cell count differed significantly between patients who achieved >200 CD4+ lymphocytes/mm³ at the end of treatment (166 + 46 cells/mm³) and those who did not achieve this threshold (88 ± 26 cells/mm³, P = 0.001), whereas age, sex, HCV/HIV co-infection and a previous AIDS-defining event did not correlate with CD4+ T-cell count at treatment end. CD8+ T-cell percentage and absolute count remained stable during the study (table 2).

Changes in T lymphocyte subpopulations

No statistically significant changes were observed in the percentage of naive and memory CD4+ T-cells over time (table 2). As T-cell counts increased during treatment, a significant increase in the absolute counts of both naive and memory CD4+ T-cells was observed at the end of treatment (data not shown). Notably, rIL-2 treatment led to a significant decrease in cell surface expression of 

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Values</th>
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<tbody>
<tr>
<td>Sex F/M (%)</td>
<td>18/4</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>43 (27-58)</td>
</tr>
<tr>
<td>Route of HIV infection (%)</td>
<td>7 (37)</td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>10 (53)</td>
</tr>
<tr>
<td>Sexual intercourse</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Transfusion</td>
<td>2 (10)</td>
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<tr>
<td>Previous AIDS diagnosis (%)</td>
<td>12 (64)</td>
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</table>
| Median plasma HIV-RNA level (copies/mL) | 106 IU daily for 5 consecutive days, every 4 weeks. Patients receiving at least one dose of rIL-2 were included in the safety analysis, based on changes in plasma HIV-RNA and laboratory parameters as well as clinical safety and tolerability. Blood samples for measuring plasma HIV-RNA, lymphocyte subset analysis, and T-cell production of intracellular cytokines were obtained on the first day of each cycle prior to dose administration, and one month after the last cycle. After completion of therapy, blood samples were obtained every three months. Efficacy was evaluated in patients receiving at least three cycles. The primary study endpoint was the median change in CD4+ lymphocyte counts at the end of treatment from baseline. Secondary endpoints were the percentage of patients who attained more than 200 CD4+ T-cells/mm³, changes in the percentile age of lymphocyte phenotype subsets, assessment of intracellular IL-2, IFN-γ, IL-4 and TNF-α production, and the tolerance and safety of rIL-2 therapy.

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Changes in T lymphocyte subpopulations

No statistically significant changes were observed in the percentage of naive and memory CD4+ T-cells over time (table 2). As T-cell counts increased during treatment, a significant increase in the absolute counts of both naive and memory CD4+ T-cells was observed at the end of treatment (data not shown). Notably, rIL-2 treatment led to a significant decrease in cell surface expression of...
TABLE 2. Changes in lymphocyte subsets over the study period

<table>
<thead>
<tr>
<th>CD4+ T cells</th>
<th>N Baseline Median (range)</th>
<th>N Cycle 3 Median (range)</th>
<th>P</th>
<th>N Cycle 6 Median (range)</th>
<th>P</th>
<th>N Follow-upb</th>
<th>Median (range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells/mm³</td>
<td>18 99 (36-195)</td>
<td>18 139 (39-225)</td>
<td>0.001</td>
<td>18 147 (24-285)</td>
<td>0.002</td>
<td>18 180 (38-280)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>CD4RA (%)</td>
<td>17 6 (0-26)</td>
<td>14 9 (0-16)</td>
<td>0.175</td>
<td>12 8 (1-23)</td>
<td>0.636</td>
<td>11 13 (3-32)</td>
<td>0.192</td>
<td></td>
</tr>
<tr>
<td>CD4R0 (%)</td>
<td>17 68 (27-91)</td>
<td>14 71 (39-90)</td>
<td>0.032</td>
<td>12 51 (13-87)</td>
<td>0.158</td>
<td>11 68 (53-82)</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>HLA-DR (%)</td>
<td>17 17 (0-74)</td>
<td>14 17 (0-74)</td>
<td>0.189</td>
<td>12 17 (8-37)</td>
<td>0.037</td>
<td>11 17 (7-53)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>CD95 (%)</td>
<td>17 72 (43-93)</td>
<td>14 71 (51-87)</td>
<td>0.682</td>
<td>12 73 (53-84)</td>
<td>0.783</td>
<td>11 77 (51-82)</td>
<td>0.790</td>
<td></td>
</tr>
<tr>
<td>CD28 (%)</td>
<td>17 78 (10-61)</td>
<td>14 76 (9-74)</td>
<td>0.005</td>
<td>12 78 (13-47)</td>
<td>0.166</td>
<td>11 78 (13-47)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>CD25 (%)</td>
<td>17 76 (27-91)</td>
<td>14 77 (76-100)</td>
<td>0.246</td>
<td>12 78 (86-100)</td>
<td>0.150</td>
<td>11 76 (50-100)</td>
<td>0.889</td>
<td></td>
</tr>
<tr>
<td>CD56 (%)</td>
<td>17 92 (80-100)</td>
<td>14 88 (54-95)</td>
<td>0.045</td>
<td>12 88 (25-95)</td>
<td>0.270</td>
<td>11 88 (68-98)</td>
<td>0.075</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>CD8+ T cells</th>
<th>N Baseline Median (range)</th>
<th>N Cycle 3 Median (range)</th>
<th>P</th>
<th>N Cycle 6 Median (range)</th>
<th>P</th>
<th>N Follow-upb</th>
<th>Median (range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells/mm³</td>
<td>18 41 (135-1700)</td>
<td>18 54 (135-1700)</td>
<td>0.117</td>
<td>18 53 (185-1775)</td>
<td>0.133</td>
<td>18 56 (177-1400)</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>CD25 (%)</td>
<td>17 17 (0-74)</td>
<td>14 17 (0-74)</td>
<td>0.964</td>
<td>12 14 (1-41)</td>
<td>0.283</td>
<td>11 13 (3-52)</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>CD28 (%)</td>
<td>17 39 (14-74)</td>
<td>14 39 (14-74)</td>
<td>0.926</td>
<td>12 38 (17-46)</td>
<td>0.053</td>
<td>11 41 (13-84)</td>
<td>0.988</td>
<td></td>
</tr>
<tr>
<td>HLA-DR (%)</td>
<td>17 42 (21-73)</td>
<td>14 38 (17-70)</td>
<td>0.084</td>
<td>12 39 (10-72)</td>
<td>0.003</td>
<td>11 34 (9-74)</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>CD95 (%)</td>
<td>17 68 (36-99)</td>
<td>14 52 (22-90)</td>
<td>0.011</td>
<td>12 46 (24-84)</td>
<td>0.020</td>
<td>11 46 (14-87)</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>CD28 (%)</td>
<td>17 49 (15-87)</td>
<td>14 53 (13-87)</td>
<td>0.258</td>
<td>12 52 (30-79)</td>
<td>0.081</td>
<td>11 48 (11-88)</td>
<td>0.612</td>
<td></td>
</tr>
<tr>
<td>CD25 (%)</td>
<td>17 4 (1-27)</td>
<td>14 5 (1-18)</td>
<td>0.152</td>
<td>12 7 (1-31)</td>
<td>0.099</td>
<td>11 3 (0-25)</td>
<td>0.237</td>
<td></td>
</tr>
<tr>
<td>CD56 (%)</td>
<td>17 51 (28-84)</td>
<td>14 58 (22-87)</td>
<td>0.404</td>
<td>12 60 (28-88)</td>
<td>0.055</td>
<td>11 60 (32-86)</td>
<td>0.062</td>
<td></td>
</tr>
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</table>

HLA-DR and CD95 on CD4+ T-cells, and HLA-DR and CD38 on CD8+ T-cells (table 2). Furthermore, there was an increase in the expression of the alpha-chain of IL-2 receptor (CD25) on CD4+ T lymphocytes, and the effector cell marker (CD28) on CD8+ T lymphocytes (table 2). A trend toward a correlation was found between the HLA-DR expression rate on CD4+ T-cells and CD4+ T-cell counts at baseline (r = -0.20, P = 0.12), and between the decrease in HLA-DR expression on CD4+ T-cells and the increase in CD4+ T lymphocyte counts observed one month after the last dose, as compared to baseline (r = 0.345, P = 0.09).

Assessment of cytokine production
The percentage of IL-2-producing CD4+ T-cells increased from a median of 9.8% (range, 3.0-31) at baseline to 22.8% (range, 3.2-32.7) after the third cycle (P = 0.003) and 27% (range, 15.5-64.1) at the end of treatment (P = 0.005). Similarly, the percentage of IL-2-producing CD4+ T-cells significantly increased at the end of treatment as compared to baseline (P = 0.003). Intracellular expression of IFN-γ, TNF-α and IL-4 in both CD4+ and CD8+ T-cells remained unchanged during the study.

Safety
Mild constitutional symptoms and local erythema at the injection site occurred in all patients. Three patients discontinued treatment because of intolerance, one of them after the first dose, and two others after the third and the fifth cycle, respectively. Only one patient suffered severe side effects. In brief, after the third dose of the fifth cycle he was admitted to another hospital because of high fever (41 °C), chest tightness and erythroderma. Oxygen and 6-methylprednisolone were administered, and the episode was definitively stopped, and he experienced a rapid clinical improvement. A transitory increase in HIV-RNA was seen in 56% of patients (9/18) during the treatment period.

Discussion
Overall, subcutaneous rIL-2 significantly increased both absolute count and percentage of the CD4+ lymphocyte pool. Our results confirm that rIL-2 might improve the immunological status of patients who start HAART with advanced HIV infection and persist with < 200 CD4+ lymphocytes/mm³, despite viral suppression to undetectable levels. However, in agreement with previous reports, individual response to rIL-2 varied widely, and almost 30% (5/18) of patients receiving at least 3 cycles of rIL-2 had a minimal or null response (CD4+ T-cell increase ≤ 25%). The lower percentage of patients who achieved 200 CD4+ T-cells/mm³ in our study in comparison with previous reports may be related to a more advanced disease status in our population, as reflected by a lower CD4+ T-cell count at baseline (99 cells/mm³) and the high rate of patients with a previous AIDS-defining clinical event (64%), or to the lower daily dose of rIL-2 administered in our study. We tried this dose, which provides the same accumulated monthly amount of IL-2 as the standard dose, because lower daily doses of rIL-2 have been proven effective, are better tolerated, and allow for a better quality of life. In keeping with previous studies, the treatment was in general well tolerated, yet one patient suffered a life-threatening event.

Poor immune reconstitution in HIV-infected patients on HAART has been related to low nadir CD4+ T-cell counts and markers of limited thymopoiesis, including older age, decreased thymic mass and smaller number of peripheral naïve CD4+ T-cells. Furthermore, persistent immune activation and spontaneous apoptosis were found to be a consistent feature in patients with a
discordant immune response, supporting a role for immune activation in the pathogenesis of CD4 T-cell depletion and immune reconstitution following HAART

Several hypotheses such as cellular proliferation, prevention of apoptosis, and neo-thymic production have been proposed to explain rIL-2-induced CD4 T-cell expansion, but the mechanisms are still not completely understood. Recently reported results suggest that intermittent rIL-2 leads to expansion of the CD4 T-cell pool by down-regulation of immune activation and T-cell proliferation rates. Additionally, in vivo labeling studies of the CD4 T-cell pool before and after rIL-2 therapy identified the emergence of a long-lived CD4 T-cell subpopulation (CD4 + CD25 + CD45RO + CD27 +) and recent memory phenotypes (CD4 + CD27 +) in vitro. In vivo study has shown that IL-2 production and CD25 expression shape a population of regulatory T-cells (CD4 + CD25 + CD62L -) that accounts for 1% to 2% of the entire CD4 T-cell pool and plays an essential role in peripheral CD4 T-cell homeostasis. Loss of these regulatory cells has been associated with higher levels of immune activation and decreases in the number of peripheral CD4 T-cells.

In keeping with these results, we observed a sustained increase of CD25 expression on CD4 + T-cells (although we did not further characterize them as regulatory cells) and a significant decrease in the expression of immune activation markers on both CD4 + and CD8 + T-cells. In addition, there was an increase in co-stimulatory molecule CD28 expression on CD8 + lymphocytes, which also suggests a decreased immune activation state. It has been shown that CD8 + T-cells lacking CD28 surface expression represent a subset of short-lived activated and differentiated cytotoxic effector cells, driven by viral replication. Indeed, the percentage of both CD4 + and CD8 + T-cells expressing intracellular IL-2 did increase significantly.

Interestingly, immunological recovery continued in our study after rIL-2 was discontinued. This long-lasting effect has been observed in patients with advanced HIV infection and can be explained by the persistence of rIL-2-driven homeostatic changes.

The main limitation of our study is the lack of a control group. The immunological improvement could be explained alternatively by persistent HAART-induced viral suppression, which could be related to a slow but ongoing decay in viremia over time. Another potential limitation is the small size of the population studied. Assessment of T lymphocyte subpopulation changes over time was only performed in a subset of patients. This fact could explain the lack of significant changes in naive and memory CD4 T-cell percentages, as well as the lack of a significant correlation between the CD4 + T-cell count increase and decreased expression of immune activation markers. Finally, although the use of rIL-2 makes particular sense in patients with advanced infection such as those included in our study, its clinical benefit in this sub-population has yet to be definitively proven.

In summary, although high interindividual variability was observed, our data suggest that rIL-2 therapy might expand the CD4 + T-cell pool in some patients with advanced disease and a blunted immune response to HAART. These rIL-2-driven changes could be mediated by down-regulation of immune activation status.

The results of the ongoing SILCAAT trial will determine whether the changes in the number and function of rIL-2-induced CD4 T-cells translate into clinical benefit.

Acknowledgments

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References


7. 250/H11350


leukin-2 therapy in addition to highly active antiretroviral therapy. AIDS. 2002;16:151-60.