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/mm$^3$ without a significant increase in the previous 12 months were scheduled to receive 6 cycles of 4.5 $\times$ 10$^6$ 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$^+$ T-cell counts were 36 and 99 cells/mm$^3$, respectively. Three patients discontinued treatment and one experienced grade 4 side effects. CD4$^+$ T-cell counts increased to 147 cells/mm$^3$ (range, 24-285) at 1 month following completion of treatment ($P = 0.002$), and 180 cells/mm$^3$ (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.

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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 2003 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 × 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 lyzed fresh whole blood samples by flow cytometry (FACScan; Becton Dickinson, San Jose, CA), and CellQuest 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+ lymphocytes

Overall, rIL-2 led to an increase in CD4+ T-cell counts from a median of 98 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, 4.19–10% (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 2 (11%) ($P = 0.501$ 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...
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-cells, and the effector cell marker (CD28) on CD8+ T-cells (table 2). A trend toward a correlation was found between the increase in CD4+ T-cell 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, 3.2-52.7) 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 CD8+ 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 (41.5 °C), chest tightness and erythroderma. Oxygen and 6-methylprednisolone were administered, rIL-2 was definitively stopped, and he experienced a rapid clinical improvement. A transitory increase in HIV-RNA was seen in 50% 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 levels²⁹. However, in agreement with previous reports⁵-⁸ may be related to a more advanced disease status in our population, as reflected by a lower nadir 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 rIL-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 life³⁰. 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 count²⁸,²⁹ and markers of limited thymopoiesis, including older age, decreased thymic mass and smaller number of peripheral naïve CD4+ T-cells²⁸,²⁹. Furthermore, persistent immune activation and spontaneous apoptosis were found to be a consistent feature in patients with a

| TABLE 2. Changes in lymphocyte subsets over the study period |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                          | N            | Baseline | Medium (range) | P       | N            | Cycle 3 | Medium (range) | P       | N            | Cycle 4 | Medium (range) | P       | N            | Follow-up | Medium (range) |
| CD4+ T-cells             |              |          |                |         |              |         |                |         |              |         |                |         |              |           |                |
| Cells/µl                 | 18           | 99 (36-195)  | 18 | 139 (38-225)  | 0.001 | 18 | 147 (24-285)  | 0.002 | 18 | 180 (38-280)  | < 0.001 |
| Percentage               | 18           | 14 (4-39)   | 18 | 23 (3-21)     | 0.007 | 18 | 15 (2-21)     | 0.004 | 18 | 13 (3-21)     | 0.003  |
| CD4RA (%)                | 17           | 6 (0-26)    | 17 | 9 (0-16)      | 0.175 | 12 | 8 (1-23)      | 0.636 | 11 | 13 (3-32)     | 0.192  |
| CD4RB (%)                | 17           | 68 (27-94)  | 17 | 71 (39-96)    | 0.352 | 12 | 57 (13-87)    | 0.138 | 11 | 69 (53-82)    | 1.185  |
| HLA-DR (%)               | 17           | 25 (8-65)   | 17 | 24 (7-65)     | 0.289 | 12 | 17 (8-37)     | 0.637 | 11 | 17 (7-53)     | 0.005  |
| CD8A (%)                 | 17           | 72 (43-93)  | 17 | 71 (51-87)    | 0.682 | 12 | 73 (53-84)    | 0.783 | 11 | 77 (51-82)    | 0.790  |
| CD28 (%)                 | 17           | 28 (10-46)  | 17 | 33 (15-61)    | 0.005 | 12 | 28 (13-47)    | 0.169 | 11 | 28 (13-47)    | 0.169  |
| CD45R0 (%)               | 17           | 98 (77-100) | 17 | 97 (78-100)   | 0.246 | 12 | 98 (86-100)   | 0.150 | 11 | 96 (50-100)   | 0.899  |
| CD45RA (%)               | 17           | 92 (80-100) | 17 | 80 (54-100)   | 0.045 | 12 | 86 (25-99)    | 0.270 | 11 | 81 (68-98)    | 0.075  |

- Eighteen months following the last dose.
- N denotes the number of patients tested.
- P = Wilcoxon signed-rank test value for comparison with baseline values. Significant P values are highlighted.

*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 rIL-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 count 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 im-
une activation in the pathogenesis of CD4+ T-cell deple-
tion and immune reconstitution following HAART14-16. Several hypotheses such as cellular proliferation21, pre-
vention of apoptosis22, and neo-epithmic production11 have been proposed to explain rIL-2-induced CD4+ T-cell expan-
sion, but the mechanisms are still not completely un-
derstood. 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 rates19. 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+) in both naïve (CD45RA+CD27+) and re-
centrated memory phenotypes (CD45RO+CD27+) in vitro. In vitro study has shown that IL-2 production and CD25 expres-
sion 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 peripher-
al CD4+ T-cell homeostasis21. Loss of these regulatory cells has been associated with higher levels of immune ac-
tivation and decreases in the number of peripheral CD4+
T-cells20. 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 regu-
ulatory) 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-stimula-
tory 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 replication21. 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 infection12,13 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 ex-
plained alternatively by persistent HAART-induced viral suppres-
sion13, which could be related to a slow but ongo-
ing decay in viremia over time. Another potential limita-
tion is the small size of the population studied. Assess-
ment 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 naïve and mem-
ory CD4+ T-cell percentages, as well as the lack of a sig-
ificant correlation between the CD4+ T-cell count in-
crease and decreased expression of immune activation markers. Finally, although the use of rIL-2 makes partic-
ular 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 translates into clinical benefit.

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