ORIGINALES BREVES

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

Manuel Crespo[a], Isabel Caragol[b], Vicenç Falcó[a], Esteban Ribera[a], Susana Urban[b] and Albert Pahissa[a]

[a]Infectious Diseases Department. [b]Immunology Unit. Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona. Spain.

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 4.5×10^6 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+ 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.

Eficacia 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 $4,5 \times 10^6$ mUI/día por vía subcutánea, cada 4 semanas, hasta completar 6 ciclos.

RESULTADOS. La edad media de los pacientes fue 43 años, el 64% con diagnóstico previo de sida. La media de linfocitos CD4+ basal (99 células/mm³) aumentó hasta 147 células/mm³ (intervalo, 24-285) al final del tratamiento (p = 0,002) y 180 células/mm³ (intervalo, 38-280) 18 meses después de la última dosis de rIL-2. Este aumento 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[1]. Several trials have shown that

TABLE 1. **Baseline characteristics of 19 HIV-infected patients with poor immune-recovery despite persistent viral suppression under HAART**

Characteristic	Values
Sex M/F	15/4
Median age, years (range)	43 (27-58)
Route of HIV infection (%)	
Intravenous drug use	7 (37)
Sexual intercourse	10 (53)
Transfusion	2 (10)
Previous AIDS diagnosis (%)	12 (64)
Median CD4 T-cell count/mm^3 (range)	
Nadir	36 (3-90)
At study enrollment	99 (36-195)
Median time on HAART, months (range)	56.5 (12-69)
Antiretroviral treatment (%)	
2 NRTI + 1 NNRTI	11 (58)
2 NRTI + 1 PI	5 (26)
3 NRTI	3 (16)
HCV/HIV co-infection, n (%)	8 (42)

M: male; F: female; HAART: highly active antiretroviral therapy; HCV: hepatitis C virus; NRTI: nucleoside reverse-transcriptase inhibitor; NNRTI: non-nucleoside reverse-transcriptase inhibitor; PI: protease inhibitor.

various schedules of recombinant interleukin-2 (rIL-2) in combination with HAART safely increase CD4+ cell counts in patients with relatively high baseline CD4+ - values[2-4]. Nevertheless, there is little data on the usefulness of rIL-2 in patients with advanced immune deficiency[5-8]. Furthermore, the impact on repopulation of various T-cell subsets, the long-term outcome of these patients, and the mechanism of rIL-2-induced immune restoration have not been completely elucidated.

This study assesses the efficacy and safety of intermittent subcutaneous rIL-2 administration in a group of patients with chronic HIV-1 infection on stable HAART and CD4+ T-cell counts persistently less than 200/mm^3 despite undetectable plasma HIV-RNA.

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/ mm^3 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 4.5×10^6 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/mm^3, changes in the percent-

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.

Laboratory testing

Lymphocyte subpopulations were analyzed in lysed fresh whole blood samples by flow cytometry (FACScalibur; 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/mm^3 (range, 36-195 cells/mm^3) to 147 cells/mm^3 (range, 24-285 cells/mm^3) after the last dose of treatment ($P = 0.002$), and 180 cells/mm^3 (range, 38-280 cells/mm^3) 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/mm^3 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/mm^3 at the end of treatment (166 ± 46 cells/mm^3) and those who did not achieve this threshold (88 ± 26 cells/mm^3; $P = 0.001$), 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 naïve 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**

	N	Baseline Median (range)	N	Cycle 3 Median (range)	P	N	Cycle 6 Median (range)	P	N	Follow-up* Median (range)	P
CD4+ T cells											
Cells/mm^3	18	99 (36-195)	18	139 (39-225)	**0.001**	18	147 (24-285)	**0.002**	18	180 (38-280)	**< 0.001**
Percentage	18	10 (4-19)	18	13 (3-21)	**0.007**	18	13 (2-21)	**0.004**	18	13 (3-21)	**0.003**
CD45RA+ (%)	17	6 (0-26)	14	9 (0-16)	0.175	12	8 (1-23)	0.636	11	13 (0-32)	0.192
CD45R0+ (%)	17	68 (27-91)	14	71 (39-90)	0.552	12	57 (19-87)	0.158	11	69 (33-82)	0.185
HLA-DR+ (%)	17	25 (8-65)	14	24 (7-65)	0.289	12	17 (8-37)	**0.037**	11	17 (7-53)	**0.005**
CD62+ (%)	17	72 (43-93)	14	71 (51-87)	0.682	12	73 (53-84)	0.783	11	77 (51-82)	0.790
CD25+ (%)	17	26 (10-46)	14	33 (15-61)	**0.005**	12	32 (14-56)	**0.008**	11	28 (13-47)	0.166
CD28+ (%)	17	98 (77-100)	14	97 (78-100)	0.246	12	98 (86-100)	0.150	11	96 (50-100)	0.889
CD95+ (%)	17	92 (80-100)	14	80 (54-100)	**0.045**	12	86 (25-99)	0.270	11	81 (68-98)	0.075
CD8+ T cells											
Cells/mm^3	18	413 (135-1700)	18	548 (165-1825)	0.117	18	553 (165-1775)	0.133	18	560 (177-1400)	0.064
Percentage	18	52 (15-72)	18	56 (15-73)	0.585	18	52 (15-71)	0.648	18	52 (16-71)	0.959
CD45RA+ (%)	17	17 (0-54)	14	19 (7-43)	0.964	12	14 (1-41)	0.283	11	19 (3-52)	0.074
CD45RO+ (%)	17	39 (14-77)	14	39 (3-69)	0.826	12	36 (17-60)	0.053	11	45 (11-81)	0.893
HLA-DR+ (%)	17	42 (21-73)	14	38 (17-70)	0.084	12	30 (10-72)	**0.003**	11	34 (9-74)	**0.008**
CD38+ (%)**	17	68 (36-99)	14	52 (22-90)	**0.011**	12	46 (24-84)	**0.020**	11	46 (14-87)	**0.013**
CD62+ (%)	17	49 (15-87)	14	53 (13-63)	0.258	12	52 (30-79)	0.081	11	48 (21-88)	0.612
CD25+ (%)	17	4 (1-27)	14	5 (1-18)	0.152	12	7 (1-31)	0.099	11	3 (0-25)	0.237
CD28+ (%)	17	51 (28-84)	14	58 (22-87)	**0.040**	12	60 (28-88)	**0.005**	11	60 (32-86)	0.062
CD95+ (%)	17	83 (70-95)	13	70 (28-99)	0.099	12	80 (46-99)	0.666	11	84 (44-99)	0.075

*Eighteen months following the last dose.
**Percentage of CD8+ cells with moderate to intense CD38 expression.
N denotes the number of patients tested. P = Wilcoxon signed-rank test value for comparison with baseline values. Significant P values are highlighted.

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 = −0.30; 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, 15.5-61.4) 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 °C), chest tightness and erythroderma. Oxygen and 6-methylprednisolone were administered, rIL-2 was definitively stopped, and he experienced a rapid clinical im-

provement. 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/mm^3, despite viral suppression to undetectable levels[5-8]. However, in agreement with previous reports[9], 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/mm^3 in our study in comparison with previous reports[5-8] 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/mm^3) 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[5]. 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[10,11] and markers of limited thymopoiesis, including older age, decreased thymic mass and smaller number of peripheral naïve CD4+ T-cells[12,13]. 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[14-16].

Several hypotheses such as cellular proliferation[17], prevention of apoptosis[18], and neo-thymic production[7] have been proposed to explain rIL-2-driven 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 downregulation of immune activation and T-cell proliferation rates[19]. 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+) on both naïve (CD45RA + CD27+) and recall memory phenotypes (CD45RO + CD27+)[20]. In vitro study has shown that IL-2 production and CD25 expression shape a population of regulatory T-cells (CD4+ CD25+ CD62+) that accounts for 1% to 2% of the entire CD4+ T-cell pool and plays an essential role in peripheral CD4+ T-cell homeostasis[21]. Loss of these regulatory cells has been associated with higher levels of immune activation and decreases in the number of peripheral CD4+ T-cells[22]. 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[23]. 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[5,7] 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[24], 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 naïve 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 subpopulation 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.

References

1. Lederman HM, Williams PL, Wu JW, Evans TG, Cohn SE, McCutchan JA, et al. Incomplete immune reconstitution after initiation of highly active antiretroviral therapy in human immunodeficiency virus-infected patients with severe CD4+ cell depletion. J Infect Dis. 2003;188:1794-803.
2. Levy Y, Durier C, Krzysiek R, Rabian C, Capitant C, Lascaux AS, et al. Effects of interleukin-2 therapy combined with highly active antiretroviral therapy on immune restoration in HIV-1 infection: a randomized controlled trial. AIDS. 2003;17:343-51.
3. Losso MH, Belloso WH, Emery S, Benetucci JA, Cahn PE, Lasala MC, et al. A randomized, controlled, phase II trial comparing escalating doses of subcutaneous interleukin-2 plus antiretrovirals versus antiretrovirals alone in human immunodeficiency virus-infected patients with CD4+ cell counts ≥ 350/mm³. J Infect Dis. 2000;181:1614-21.
4. Davey RT, Jr., Murphy RL, Graziano FM, Boswell SL, Pavia AT, Cancio M, et al. Immunologic and virologic effects of subcutaneous interleukin 2 in combination with antiretroviral therapy: A randomized controlled trial. JAMA. 2000;284:183-9.
5. Arnó A, Ruiz L, Juan M, Jon A, Balagué M, Zayat MK, et al. Efficacy of low-dose subcutaneous interleukin-2 to treat advanced human immunodeficiency virus type 1 in persons with ≤ 250/µL CD4 T cells and undetectable plasma virus load. J Infect Dis. 1999;180:56-60.
6. David D, Nait-Ighil L, Dupont B, Maral J, Gachot B, Theze J. 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. J Infect Dis. 2001;183:730-5.
7. Marchetti G, Meroni L, Varchetta S, Terzieva V, Bandera A, Manganaro D, et al. Low-dose prolonged intermittent interleukin-2 adjuvant therapy: results of a randomized trial among human immunodeficiency virus-positive patients with advanced immune impairment. J Infect Dis. 2002;186:606-16.
8. Katlama C, Carcelain G, Duvivier C, Chouquet C, Tubiana R, De Sa M, et al. Interleukin-2 accelerates CD4 cell reconstitution in HIV-infected patients with severe immunosuppression despite highly active antiretroviral therapy: the ILSTIM study – ANRS 082. AIDS. 2002;16:2027-34.
9. Farel CE, Chaitt DG, Hahn BK, Tavel JA, Kovacs JA, Polis MA, et al. Induction and maintenance therapy with intermittent interleukin-2 in HIV-1 infection. Blood. 2004;103:3282-6.
10. Renaud M, Katlama C, Mallet A, Calvez V, Carcelain G, Tubiana R, et al. Determinants of paradoxical CD4 cell reconstitution after protease inhibitor-containing antiretroviral regimen. AIDS. 1999;13:669-76.
11. García F, de Lazzari LE, Plana M, Castro P, Mestre G, Nomdedeu M, et al. Long-term CD4+ T-cell response to highly active antiretroviral therapy according to baseline CD4+ T-cell count. J Acquir Immune Defic Syndr. 2004; 36:702-13.
12. Notermans DW, Pakker NG, Hamann D, Fondraine NA, Kauffmann RH, Meenhorst PL, et al. Immune reconstitution after 2 years of successful potent antiretroviral therapy in previously untreated human immunodeficiency virus type 1-infected adults. J Infect Dis. 1999;180:1050-6.
13. Kalayjian RC, Spritzler J, Pu M, Landay A, Pollard RB, Stocker V, et al. Distinct mechanisms of T cell reconstitution can be identified by estimating thymic volume in adult HIV-1 disease. J Infect Dis. 2005;192:1577-87.
14. Benveniste O, Flahault A, Rollot F, Elbim C, Estaquier J, Pédron B, et al. Mechanisms involved in the low-level regeneration of CD4+ cells in HIV-1-infected patients receiving highly active antiretroviral therapy who have prolonged undetectable plasma viral loads. J Infect Dis. 2005;191: 1670-9.
15. Hansjee N, Kaufmann GR, Strub C, Weber R, Battegay M, Erb P. Persistent apoptosis in HIV-1-infected individuals receiving potent antiretroviral therapy is associated with poor recovery of CD4 T lymphocytes. J Acquir Immune Defic Syndr. 2004;36:671-7.
16. Anthony KB, Yoder C, Metcalf JA, Der Simonian R, Orenstein JM, Stevens RA, et al. Incomplete CD4 T cell recovery in HIV-1 infection after 12 months of highly active antiretroviral therapy is associated with ongoing increased CD4 T cell activation and turnover. J Acquir Immune Defic Syndr. 2003; 33:125-33.
17. Hengge UR, Borchard C, Esser S, Schroder M, Mirmohammadsadegh A, Goos M. Lymphocytes proliferate in blood and lymph nodes following inter-

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

18. Pandolfi F, Pierdominici M, Marziali M, Liria Bernardi M, Antonelli G, Galati V, et al. Low-dose IL-2 reduces lymphocyte apoptosis and increases naive CD4 cells in HIV-1 patients treated with HAART. Clin Immunol. 2000; 94:153-9.

19. Sereti I, Anthony KB, Martinez-Wilson H, Lempicki R, Adelsberger J, Metcalf JA, et al. IL-2-induced CD4+ T-cell expansion in HIV-infected patients is associated with long-term decreases in T-cell proliferation. Blood. 2004; 104:775-80.

20. De Paoli PP, Zanussi S, Simonelli C, Bortolin MT, D'Andrea M, Crepaldi C, et al. Effects of subcutaneous interleukin-2 therapy on CD4 subsets and in vitro cytokine production in HIV+ subjects. J Clin Invest. 1997;100: 2737-43.

21. Almeida AR, Legrand N, Papiernik M, Freitas AA. Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. J Immunol. 2002;169:4850-60.

22. Eggena MP, Barugahare B, Jones N, Okello M, Mutalya S, Kityo C, et al. Depletion of regulatory T cells in HIV infection is associated with immune activation. J Immunol. 2005;174:4407-14.

23. Borthwick NJ, Bofill M, Gombert WM, Akbar AN, Medina E, Sagawa K, et al. Lymphocyte activation in HIV-1 infection. II. Functional defects of CD28- T cells. AIDS. 1994;8:431-41.

24. Koletar SL, Williams PL, Wu J, McCutchan JA, Cohn SE, Murphy RL, et al. Long-term follow-up of HIV-infected individuals who have significant increases in CD4+ cell counts during antiretroviral therapy. Clin Infect Dis. 2004;39:1500-6.