

IL-2–induced CD4⁺ T-cell expansion in HIV-infected patients is associated with long-term decreases in T-cell proliferation

Irini Sereti, Kara B. Anthony, Hector Martinez-Wilson, Richard Lempicki, Joseph Adelsberger, Julia A. Metcalf, Claire W. Hallahan, Dean Follmann, Richard T. Davey, Joseph A. Kovacs, and H. Clifford Lane

Administration of interleukin 2 (IL-2) leads to selective and sustained CD4⁺ T-cell expansions in patients infected with HIV. It has been hypothesized that persistent CD4⁺ T-cell proliferation is the primary mechanism maintaining these expansions. T-cell proliferation was studied by ex vivo bromodeoxyuridine (BrdU) incorporation and intracellular Ki67 staining in HIV-infected patients treated with antiretroviral therapy (ART) with or without IL-2. In contrast to the tested hypothesis, HIV-

infected patients treated with IL-2 had lower CD4⁺ T-cell proliferation compared to patients treated with ART alone. Independently of viral load changes, administration of IL-2 led to a decrease in basal CD4⁺ T-cell proliferation. Total numbers of CD4⁺ T cells with naive and recall, but not effector, memory phenotype were increased. The degree of CD4⁺ T-cell expansion correlated with the decreases in proliferation and a strong association was seen between these decreases and the

expansion of the CD4⁺/CD25⁺ subset. Intermittent IL-2 in HIV-infected patients leads to expansions of CD4⁺/CD25⁺ T cells with naive and recall memory phenotypes that strongly correlate with decreases in proliferation. These data suggest that decreased T-cell proliferation is central in the CD4⁺ T-cell expansions induced by IL-2. (Blood. 2004;104:775-780)

© 2004 by The American Society of Hematology

Introduction

Administration of intermittent cycles of interleukin 2 (IL-2) to patients infected with HIV-1 leads to substantial and sustained CD4⁺ T-cell expansions.^{1,2} The initial induction regimen consists of 5-day cycles that are administered every 8 weeks for 3 to 6 cycles. The majority of treated patients then require only infrequent cycling for maintenance of these CD4 expansions.^{3,4} Studies with the use of ex vivo and in vivo labeling techniques have shown that significant proliferation of both CD4⁺ and CD8⁺ T cells occurs during IL-2 administration. Ultimately, selective expansion of only the CD4⁺ T-cell pool is observed.^{5,6} It has also been reported that intermittent IL-2 leads to a preferential long-term increase of CD4⁺ T cells expressing CD25, the α chain of the IL-2 receptor.^{3,4} These observations have led to the hypothesis that IL-2 may be preferentially sustaining CD4⁺ T-cell expansions via continued proliferation, a mechanism that contrasts with the mechanism described for antiretroviral therapy (ART). It is currently well established that HIV infection leads to a state of heightened immune activation and increased T-cell turnover.⁷⁻⁹ In studies with in vivo labeling techniques, the degree of T-cell turnover was abnormally high compared to HIV-seronegative controls and correlated with plasma viremia levels,^{10,11} with highly active antiretroviral therapy (HAART) administration leading to viral suppression and a lowering of T-cell turnover, although not always to normal levels. In the current study the long-term effects of intermittent IL-2 therapy to T-cell turnover were tested by studying the proliferation of CD4⁺ T

cells of patients treated with IL-2 in comparison with patients treated with ART alone.

Patients, materials, and methods

Patients

Cohort 1. A total of 82 consecutive patients infected with HIV-1 who agreed to participate and signed informed consent were included in this study between December 1997 and June 1998. All participants were followed at the National Institute of Allergy and Infectious Diseases/Clinical Center (NIAID/CC) clinic participating in Institutional Review Board (IRB)–approved protocols. The cohort included 40 patients (control group) who were not receiving IL-2 and were either receiving ART (35 patients) or were ART naive (5 patients), and 42 patients treated with intermittent cycles of subcutaneous IL-2 at doses of 4.5 to 7.5 million IU (MIU) 2 times a day for 5 days (IL-2 group) in combination with ART. All patients on therapy had received 3 months or longer of HAART and the majority of patients in both groups were receiving a protease inhibitor-based regimen. The characteristics of the participants (at the time of study) are shown in Table 1.

Cohort 2. Cryopreserved peripheral blood mononuclear cells (PBMCs) from 53 HIV-1–infected patients participating in a randomized, controlled, phase 2, IRB-approved protocol at the National Institutes of Health Clinical Center (NIH-CC) were used. The study design and the results of the clinical trial have been published previously.¹ Briefly, the study compared the CD4⁺

From the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; Science Applications International Corp, National Cancer Institute, Frederick, MD; and Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD

Submitted December 22, 2003; accepted March 26, 2004. Prepublished online as *Blood* First Edition Paper, April 13, 2004; DOI 10.1182/blood-2003-12-4355.

Supported in part with federal funds under National Cancer Institute contract no. NO1-CO-56000. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply

endorsement by the US government. The US government has been granted a use patent for intermittent IL-2 therapy including H.C.L. and J.A.K. as inventors.

Reprints: Irini Sereti, Clinical and Molecular Retrovirology Section, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg 10, Rm 11B-04, 10 Center Dr, MSC 1876, Bethesda, MD 20892; e-mail: isereti@niaid.nih.gov.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2004 by The American Society of Hematology

Table 1. Characteristics of participants in cohort 1

Characteristics	IL-2 group, n = 42	Control group, n = 40
No. CD4 cells/ μ L (range)	696 (108-1297)*	328 (80-962)
No. CD8 cells/ μ L (range)	1137 (437-2995)†	750 (424-2404)
No. HIV-RNA log copies/mL (range)	3.0 (2.7-5.4)	2.8 (2.7-5.6)
No. patients with fewer than 500 HIV-RNA copies/mL (%)	18/42 (43)	20/40 (50)
HIV diagnosis, y (range)	10 (2-15)‡	3 (0.5-15)
Percent CD4 T cells BrdU ⁺ (range)	0.141 (0.022-0.765)*	0.209 (0.048-1.542)
Percent CD8 T cells BrdU ⁺ (range)	0.088 (0-0.530)	0.129 (0.02-1.086)
Mos. since last IL-2 cycle (range)	5 (1-22)	NA
Total no. of IL-2 cycles (range)	11 (3-24)	NA

Median values are given, with range in parentheses.

NA indicates not applicable.

* $P < .01$ compared to control group.

† $P = .02$ compared to control group.

‡ $P < .001$ compared to control group.

T-cell counts in 2 groups of participants who had more than 200 CD4⁺ T cells/ μ L at baseline: a control group receiving nucleoside analogues alone and an IL-2 group receiving nucleoside analogues with intermittent cycles of IL-2. At baseline (month 0), there were no significant differences between the 2 groups and viral loads did not change significantly during the study in either group (Table 2). The IL-2 group participants received 5-day continuous intravenous infusions of IL-2 at 18 MIU per day every 8 weeks for a total of 3 to 6 cycles. The end of study time point (month 12 or 14) was 2 to 6 months after the last administered IL-2 cycle. Twenty-nine of 31 patients from the IL-2 group and 24 of 29 from the control group were studied. Patient selection was based on availability of stored cryopreserved viable PBMCs at month 0 and month 12 or 14. One patient from the control group was excluded from the analysis because he had received IL-2 during the study. Baseline (month 0) and end of study (month 12) characteristics of cohort 2 are shown in Table 2.

Flow cytometry

Ex vivo bromodeoxyuridine (BrdU) staining was performed as previously described.⁸

Immunophenotypic analysis of cryopreserved PBMCs was performed using 4-color immunofluorescence as previously described⁹ using the following monoclonal antibodies: CD4-peridinin chlorophyll protein (PerCP; clone SK7), CD8-PerCP (clone SK1), CD45RO-phycoerythrin (PE) or allophycocyanin (APC; clone UCHL-1), and CD27-fluorescein isothiocyanate (FITC; clone M-T271), all from BD/PharMingen Immunocytometry (San Jose, CA). Intracellular staining for the nuclear antigen Ki67 was performed using Ki67-PE (clone B56) and mouse IgG1-PE (clone MOPC-21) from BD/PharMingen. T-cell proliferation was defined as the percent of cells expressing Ki67. We have previously shown that there is a good correlation between Ki67 staining and in vivo and ex vivo BrdU staining.¹¹ Additionally, a strong correlation between Ki67 expression and surface staining with HLA-DR and CD38 in CD4⁺ and CD8⁺ T cells of HIV-infected patients has been described, so that intracellular Ki67 staining is also a good marker of immune activation.¹² To study naive and memory phenotypes, cells were stained with surface CD3, CD4 or CD8, CD45RO, and CD27 antibodies, markers that are known to remain stable with cryopreservation. Naive cells were defined as CD45RO⁻CD27⁺. Memory cells were either defined as CD45RO⁺CD27⁺ (recall or central memory) or

CD45RO⁺CD27⁻ (effector memory). In the CD8 subset, a third population of memory cells was defined as CD45RO⁻27⁻ (CD45RO⁻ memory). Samples were collected on a fluorescence-activated cell sorting (FACS) Calibur (BD Immunocytometry) using CellQuest software. Approximately 1.5 to 2×10^5 total events with a minimum of 5000 events in the CD4⁺ or CD8⁺ gate were collected per sample. Flow cytometry data were analyzed using FlowJo software (Tree Star, San Carlos, CA).

Statistical methods

Medians and distributions of the data for the 2 treatment groups were compared by the Wilcoxon 2-sample method. The median changes from baseline to month 12 were tested for significance by the Wilcoxon signed rank test. IL-2 and control group Pearson correlations were tested for equality by the Fisher z transformation for comparison of 2 independent correlations. Association between other variables was determined by the Spearman rank correlation. Linear regression and analysis of covariance were also used to quantify the relationship between variables. Adjustment of P values for multiple testing was done by the Bonferroni method.

Results

Patients with HIV treated with IL-2 have a lower basal proliferation rate of CD4⁺ T cells compared to patients who are not receiving IL-2 despite similar HIV viral loads

A cross-sectional analysis was performed of 82 patients treated with either combination ART without (40, control group) or with intermittent IL-2 (42, IL-2 group) at a single time point. The IL-2 group patients had a significantly higher CD4⁺ T-cell count and a longer time since HIV diagnosis compared to the control group (Table 1). Proliferation of CD4⁺ T cells as measured by ex vivo BrdU incorporation was lower in the IL-2 group compared to the control group ($P < .01$; Table 1). An association of CD4⁺ T-cell proliferation with viral load was noted in the control group ($r = 0.68$, $P < .001$) as well as the IL-2 group ($r = 0.57$, $P < .001$) as shown in Figure 1. By analysis of covariance, the slopes of the regression lines for the IL-2 and control groups were not different ($P = .33$) and therefore the regression lines were parallel differing by a constant amount over the tested viral load range; by this same method the intercept for the IL-2 group was determined to be statistically lower ($P < .001$) indicating that CD4⁺ T-cell proliferation was lower in the IL-2 group compared to the control group by a constant amount over the entire range of viral load. When the CD4⁺ T-cell count was statistically controlled for, by adding it to the regression model, the intercept or difference in the parallel regression lines was no longer significant ($P = .15$) indicating that the direct effect of viral load on CD4⁺ T-cell turnover was similar in the IL-2 group compared to the control group. There was no correlation between the years since diagnosis of infection and the CD4⁺ or CD8⁺ T-cell proliferation in either group ($P > .3$). An inverse correlation was noted between years of infection and the CD4⁺ T-cell count in the control group only ($r = -0.41$, $P = .02$).

Table 2. Characteristics of participants in cohort 2

Characteristics	IL-2 group (n = 29)		Control group (n = 23)	
	Month 0	Month 12	Month 0	Month 12
No. CD4 cells/ μ L (range)	416 (225-753)	713* (204-2862)	378 (206-926)	349 (56-958)
No. CD8 cells/ μ L (range)	973 (184-1786)	856 (200-2426)	866 (488-2127)	844 (329-1661)
No. HIV-RNA log copies/mL	3.55 (1.69-4.84)	3.51 (1.69-4.68)	3.74 (1.73-4.98)	3.73 (2.29-5.12)

Median values are given, with range in parentheses. * $P < .001$ compared to month 0; $P = .002$ compared to control group at month 12.

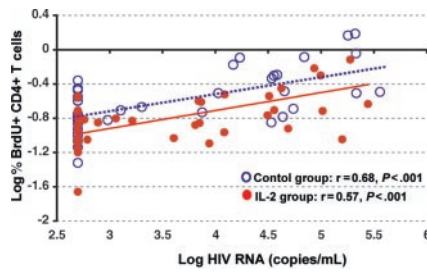


Figure 1. HIV-1-infected patients treated with ART and IL-2 have lower basal proliferation of CD4⁺ T cells compared to patients treated with ART alone. The correlation between plasma HIV-RNA and CD4⁺ T cells positive for BrdU is shown for participants treated with ART alone (open blue circles) and for IL-2 recipients (red circles). The significant difference in CD4⁺ T cell proliferation over the range of viral load in the IL-2 and control groups ($P < .001$) was not observed following adjustment for CD4⁺ T cell counts ($P = .15$).

IL-2 administration leads to increases in naive and recall memory CD4⁺ T cells but not effector cells

To better characterize these observations, and to better define the subpopulations of cells that are expanded during IL-2 therapy, a longitudinal analysis was performed of patients participating in a randomized controlled trial of IL-2.

It is known that IL-2 administration leads to a preferential increase of naive CD4⁺ T cells, although increases in memory cells are also seen.^{2,4} In this study, a statistically significant increase was seen in the naive and recall memory CD4⁺ T cells of the IL-2 group at month 12 compared to month 0, and also compared to the control group at month 12 (Figure 2A-B; $P < .001$ for all comparisons). No changes from baseline were observed in the effector memory pool. The median change of the percent of effector memory CD4⁺ T cells was significantly different between the 2 groups with a decrease of 8.70% in the IL-2 group compared to a decrease of 0.10% in the control group ($P = .002$).

In the CD8⁺ T-cell subset, no statistically significant changes from month 0 were observed in the numbers of CD8⁺ T-cell subsets in either group (Figure 2C-D). The median change in the percent of recall memory CD8⁺ T cells (+2.20% in the IL-2 group versus -3.80% in the control group) and the median change of the effector memory CD8⁺ T cells (-1.90% in the IL-2 group versus +2.60% in the control group) were also significantly different between the 2 groups ($P = .002$). This observation is in agreement

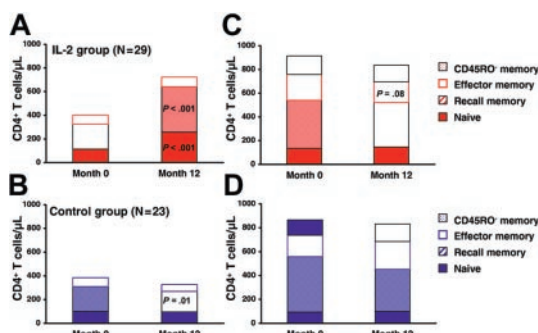


Figure 2. IL-2 administration leads to increases in naive and recall memory but not effector memory CD4⁺ T cells. The median counts of CD4⁺ (A-B) and CD8⁺ (C-D) T-cell subsets from the IL-2 and control groups from cohort 2 are shown at month 0 (baseline) and month 12. In IL-2 recipients, significant increases in the naive and recall memory CD4 subsets ($P < .001$) were observed compared to month 0 values (paired analysis) and when they were compared to controls at month 12. No statistically significant changes were noted in the control group at month 12 compared to month 0, except for a significant decrease in recall memory CD4⁺ T cells ($P = .01$).

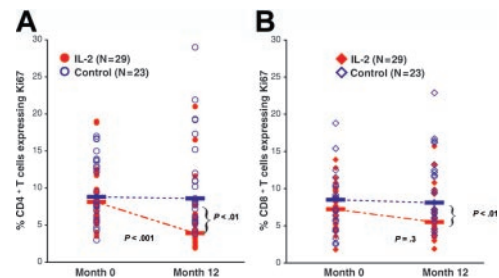


Figure 3. IL-2 decreases long-term proliferation in CD4⁺ and CD8⁺ T cells. Paired percentages of CD4⁺ (A) and CD8⁺ (B) T cells expressing Ki67 at months 0 and 12 from each individual participant from cohort 2 are shown. Solid symbols represent values from IL-2 group patients and open symbols represent measurements from the control group. No significant differences in Ki67 expression of CD4⁺ or CD8⁺ T cells were noted between the IL-2 and the control groups at month 0. At month 12, a significant difference was found between the 2 groups in the percent Ki67 expression in both CD4⁺ and CD8⁺ T cells ($P < .01$ for both comparisons). A significant decrease at month 12 compared to month 0 was found in the expression of Ki67 in the CD4⁺ T cells from the IL-2 group only ($P < .001$).

with recent reports showing decreases of CD8⁺/CD38⁺ T cells as well as rises of naive CD8⁺ T cells after IL-2 treatment.⁴

IL-2 administration leads to decreased Ki67 expression in CD4⁺ T cells

No significant differences in intracellular Ki67 expression in CD4⁺ and CD8⁺ T cells were seen at study entry (month 0) between the 2 groups. However, a statistically significant decrease in Ki67 expression in CD4⁺ T cells was noted at month 12 in the IL-2 group (Figure 3, $P < .001$), whereas no significant changes were observed in the controls. Although an overall drop was also detected in the Ki67 expression of the CD8⁺ T cells in the IL-2 group, this did not reach statistical significance in the paired analysis possibly due to considerable variability among patients. However, the percent CD8⁺ T cells expressing Ki67 at month 12 was significantly lower in the IL-2 compared to the control group ($P < .01$). In examining naive and memory subsets of T cells, statistically significant decreases in Ki67 expression were seen in naive and recall memory subsets of CD4⁺ T cells ($P = .004$ and $P < .001$, respectively) but not in the effector memory subset (Figure 4). A significant decrease was also noted in the effector memory CD8 subset ($P = .05$). No significant changes occurred in any of the subsets in the control group (data not shown).

T-cell activation was also measured in a subset of patients (18 from the IL-2 group and 12 from the controls) with HLA-DR and CD38 coexpression. Surface coexpression of HLA-DR and CD38 on T cells correlates strongly with intracellular Ki67 expression in both ART naive and ART-treated HIV-infected patients.¹² A statistically significant change at month 12 compared to month 0

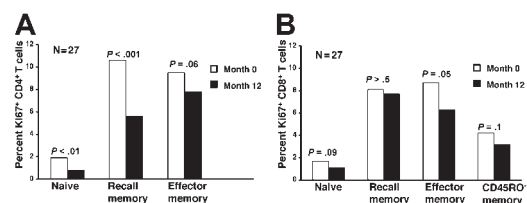


Figure 4. IL-2-induced decreases in proliferation were observed in naive and recall memory CD4⁺ T cells. Median values of percent Ki67 expression in naive and memory subsets of CD4⁺ (A) and CD8⁺ (B) T cells at baseline (□) and month 12 (■) from the IL-2 group are shown. Statistically significant decreases of Ki67 expression were noted in the naive ($P = .004$) and recall memory ($P < .001$) CD4⁺ T-cell subsets. Trends were noted in the effector CD4⁺ ($P = .06$) and CD8⁺ T cell pools ($P = .05$).

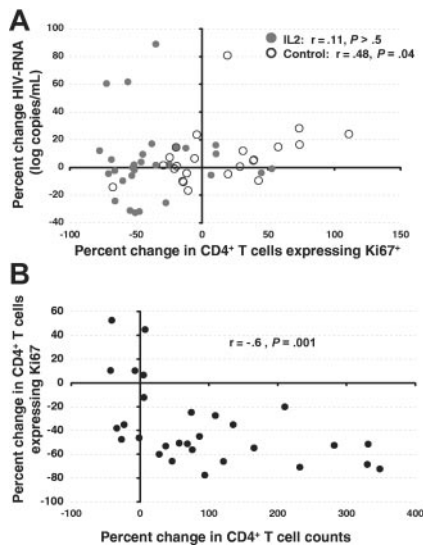


Figure 5. IL-2 led to decreases in CD4⁺ T-cell proliferation that were independent of changes in viral load and correlated with increases in the numbers of CD4⁺ T cells. (A) The correlation between changes in Ki67 expression in CD4⁺ T cells and changes in viral load is shown for the IL-2 group (●; $r = 0.11$, $P > .5$) and the control group (○; $r = 0.48$, $P = .04$). (B) A statistically significant correlation was seen between changes in CD4⁺ T-cell counts and changes in Ki67 expression in CD4⁺ T cells in the IL-2 group (Spearman correlation coefficient $r = -0.6$, $P = .001$).

was noted in the percent of CD4⁺ T cells coexpressing HLA-DR and CD38 in the IL-2 group compared to controls (-5.5 versus $+1.90$, $P < .01$). The median change of the percent of CD8⁺ T cells coexpressing HLA-DR and CD38 was also significantly different between groups (-2.70 in the IL-2 group versus $+4.65$ in the control group, $P = .003$, data not shown).

Changes in CD4⁺ T cell basal proliferation were independent of viral load changes in patients treated with IL-2

The relationship between viral load and proliferation was studied in the 2 groups from the randomized cohort. Consistent with previous reports, an association between CD4⁺ and CD8⁺ T-cell proliferation and viral load was found at study entry in both groups (data not shown). This was preserved at the end of study only in the control group and changes of viral load in the control group correlated with changes in T-cell proliferation (Figure 5, $r = 0.48$, $P = .04$). In contrast, viral load changes in the IL-2 group were not associated with changes in the proliferation of CD4⁺ T cells (Figure 5, $r = 0.11$, $P > .5$). There was a significant correlation between the

CD4⁺ and CD8⁺ T-cell proliferation in both groups at both month 0 and month 12 time points ($P < .01$, data not shown).

The expansion of CD4⁺/CD25⁺ T cells after IL-2 administration was strongly associated with decreases in turnover of this subset

Several studies have clearly shown that IL-2 leads to a significant increase of CD4⁺ T cells expressing CD25.^{1,13,14} The same observation was made in this study. In the IL-2 group the median percent of CD4⁺ T cells expressing CD25 increased from 19.6% to 53% and the CD4⁺/CD25⁺ T-cell count increased from 89 cells/ μ L to 360 cells/ μ L ($P < .001$). The CD4⁺/CD25⁻ count did not change significantly in the IL-2 group (from 334 cells/ μ L to 308 cells/ μ L, $P = .7$). No significant changes were seen in the control group in either subset (data not shown). CD25 is considered a marker of activation and the expansion of CD25-expressing CD4⁺ T cells shown earlier was paradoxical given the observed decreases in Ki67 expression in the CD4⁺ T-cell pool. To better understand this observation we studied Ki67 expression in CD4⁺/CD25⁺ and CD4⁺/CD25⁻ cells at month 0 and month 12 (Figure 6A). In the IL-2 group the median Ki67 expression in CD4⁺/CD25⁺ cells decreased significantly from 8% to 3% (Figure 6B, $P < .001$). No significant change in Ki67 expression was observed in the CD4⁺/CD25⁻ cells (Figure 6C). No changes were seen in either subset in the control group. The decrease in expression of Ki67 strongly correlated with the expansion of this subset (Figure 6B). In agreement with our previous report,³ the CD4⁺/CD25⁺ cells in the IL-2 group at month 12 were predominantly of naive or recall memory phenotype (data not shown).

Discussion

This study has demonstrated that contrary to expectations, IL-2 administration to patients infected with HIV-1 leads to long-term decreases in CD4⁺ T-cell proliferation. These decreases occurred in the naive, recall memory and CD25⁺ CD4 subsets, were strongly correlated with the observed cell increases in these pools of cells, and were independent of changes in viral load. These data suggest that decreases in proliferation and immune activation are critical in sustaining the long-lived CD4⁺ T-cell expansions that are seen in the setting of IL-2 administration.

Immune activation has been implicated as an independent factor affecting HIV disease progression.^{15,16} It has been hypothesized that persistent immune activation supports increased T-cell turnover leading to increased apoptosis and immunologic senescence

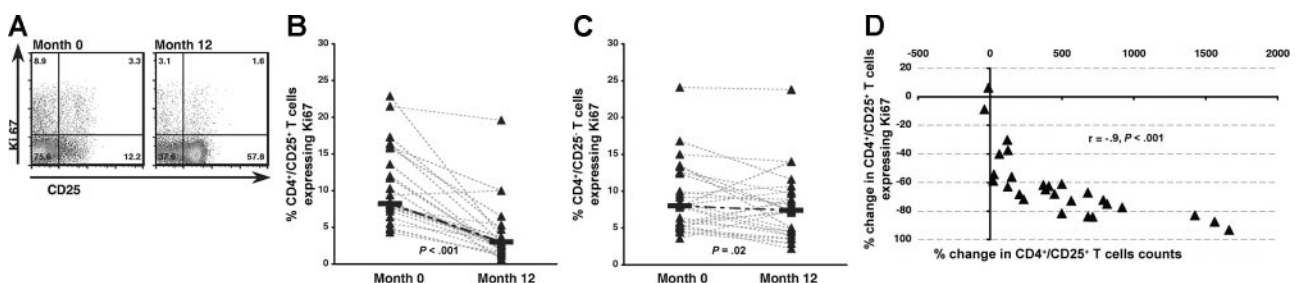


Figure 6. IL-2 induced decreases in proliferation of the CD4⁺/CD25⁺ subset that were strongly correlated with the expansion of this subset. (A) Representative density plot of CD4⁺ T cells at month 0 and month 12 from an IL-2 group patient, examining surface expression of CD25 and intracellular expression of Ki67. The striking increase of the CD4⁺/CD25⁺ subset at month 12 can be noted. (B) Paired data and median percent expression of Ki67 in CD4⁺/CD25⁺ T cells in the IL-2 group at month 0 and month 12. A statistically significant decrease in Ki67 expression was noted ($P < .001$) at month 12 compared to month 0. (C) No statistically significant changes were observed in Ki67 expression in the CD4⁺/CD25⁻ T cells. (D) A strong association was found between the decreases in Ki67 expression of the CD4⁺/CD25⁺ T cells and the increases in the number of these cells (Spearman correlation, $r = -0.9$, $P < .001$).

as well as preferential loss of naive cells with accumulation of cells with effector, terminally differentiated phenotype.¹⁶⁻¹⁸ Similar immunophenotypic changes have also been described in HIV seronegative persons with persistent immune activation due to other chronic infections.^{19,20}

Several recent studies support the hypothesis that immune activation is a primary immunopathogenic mechanism underlying the loss of CD4⁺ T cells in HIV infection. In an animal model, Silvestri and colleagues²¹ described how sooty mangabeys that remain healthy with normal life spans and normal CD4⁺ T-cell counts after simian immunodeficiency virus (SIV) infection have only minimal evidence of immune activation (slight increases in percent of CD4⁺ T cells expressing Ki67) despite high levels of SIV viremia. Sousa et al²² reported a cross-sectional analysis of patients infected with HIV-1 and HIV-2 and showed that at similar degrees of CD4⁺ T-cell lymphopenia, HIV-2-infected patients had equally elevated CD4⁺ T-cell turnover (measured by Ki67) and loss of naive T cells compared to patients infected with HIV-1 despite significantly lower levels of viremia. Interestingly, higher levels of immune activation even before HIV seroconversion have also been reported to correlate with faster AIDS progression after HIV infection in the Amsterdam cohort.²³ Persistently high turnover after combination ART initiation despite virologic success (viral load less than the limit of detection) has been associated with incomplete CD4⁺ T-cell recovery.¹² Similarly, patients with suboptimal virologic control but high CD4⁺ T-cell counts were found to have lower levels of immune activation compared to patients with similar viral loads but low CD4⁺ T-cell counts.²⁴ Taken together, these data highlight the important role of immune activation in CD4⁺ T-cell lymphopenia.

Based on these observations, therapies targeting immune activation such as corticosteroids, cyclosporine, or mycophenolate mofetil have been attempted in HIV infection²⁵⁻²⁷ and in some instances have led to at least temporary CD4⁺ T-cell increases. Although some of these efforts have been tempered due to significant toxicity²⁸ and concerns over viral load increases²⁶ or additional immunosuppression in patients with preexistent defective cellular immunity, this remains an area of active investigation.

This study clearly demonstrated that IL-2 lowered immune activation and CD4⁺ T-cell proliferation and this effect was tightly connected to CD4⁺ T-cell increases. This "normalization" of T-cell turnover, even in the presence of detectable viremia, brought about by direct manipulation of the immune system offers a new perspective on the role of IL-2 in the treatment of patients with HIV infection. This observation helps explain the lack of effect of IL-2 on viral load^{29,30} and the fact that CD4⁺ T-cell increases have been observed with IL-2 even in the absence of ART.³¹ Interestingly, despite significant acute immunostimulatory effects during the

5-day period of IL-2 administration³² that were associated with transient viral bursts in the pre-HAART era,³³ IL-2 immunotherapy in HIV infection actually appears to act by decreasing immune activation and T-cell proliferation.

Our results from the cross-sectional study of patients treated with HAART receiving subcutaneous IL-2 supported the findings of the longitudinal study of patients receiving nucleoside analogues with intravenous IL-2. In the HAART-treated cohort, a lower CD4⁺ T-cell proliferation was observed in patients treated with IL-2 compared to controls in a wide (3 log) range of viral load. Interestingly, this difference seemed to depend on the higher CD4⁺ T-cell counts of the IL-2 recipients, and thus correlated with the desired biologic effect of intermittent IL-2 immunotherapy. Although an alternative interpretation could be that different infection times in the 2 groups led to these findings, the IL-2 group had a significantly longer time since diagnosis. This is presumably a close surrogate for time of infection. Additionally, the longitudinal randomized study confirmed these findings in a group of patients with suboptimal virologic control, where the added effect of viral load on T-cell proliferation could also be studied. In a randomized study testing the use of corticosteroids to improve the tolerance of IL-2 cycles, in patients receiving HAART, it was also found that the blunted CD4⁺ T-cell expansions in the patients treated with steroids and IL-2 were associated with smaller Ki67 decreases in the CD4⁺ T cell pool compared to the group that received IL-2 alone.³⁴

The fact that IL-2 leads to rises of specific subsets of CD4⁺ T cells, naive and recall memory, without affecting the effector pool is most relevant for its potential clinical applications given the recently described dichotomy of functional characteristics of memory T cells.^{35,36} Our results suggest that although no beneficial effect toward active infections should be anticipated, one may expect improvement of host defenses against recall or neoantigens and that the timing of IL-2 with respect to immunizations should be carefully designed. Considering the fact that immunologic challenges with remote recall or neoantigens are infrequent in adults, the clinical efficacy of this approach will take time to establish. Phase 3 clinical trials addressing this question³⁷ are ongoing with results anticipated in 4 to 5 years.

In summary, intermittent IL-2 administration to HIV-infected patients decreased activation and proliferation of CD4⁺ T cells. These decreases were observed in the naive, recall memory, and CD25⁺ CD4⁺ T-cell subsets, were independent of viral load changes, and correlated strongly with the degree of CD4⁺ T-cell expansions. These data suggest that intermittent IL-2 treatment in HIV infection could be viewed predominantly as immunotherapy targeting and reversing increased T-cell proliferation and immune activation.

References

- Kovacs JA, Vogel S, Albert JM, et al. Controlled trial of interleukin-2 infusions in patients infected with the human immunodeficiency virus. *N Engl J Med*. 1996;335:1350-1356.
- Davey RT Jr, Murphy RL, Graziano FM, et al. Immunologic and virologic effects of subcutaneous interleukin 2 in combination with antiretroviral therapy: a randomized controlled trial. *JAMA*. 2000;284:183-189.
- Sereti I, Martinez-Wilson H, Metcalf JA, et al. Long-term effects of intermittent interleukin 2 therapy in patients with HIV infection: characterization of a novel subset of CD4(+)/CD25(+) T cells. *Blood*. 2002;100:2159-2167.
- Levy Y, Durier C, Krzysiek R, 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;17:343-351.
- Hengge UR, Borchard C, Esser S, Schroder M, Mirmohammadsadeh A, Goos M. Lymphocytes proliferate in blood and lymph nodes following interleukin-2 therapy in addition to highly active antiretroviral therapy. *AIDS*. 2002;16:151-160.
- Natarajan V, Lempicki RA, Sereti I, et al. Increased peripheral expansion of naive CD4⁺ T cells in vivo after IL-2 treatment of patients with HIV infection. *Proc Natl Acad Sci U S A*. 2002;99:10712-10717.
- Sachsenberg N, Perelson AS, Yerly S, et al. Turnover of CD4⁺ and CD8⁺ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. *J Exp Med*. 1998;187:1295-1303.
- Lempicki RA, Kovacs JA, Baseler MW, et al. Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of CD4⁺ and CD8⁺ T cell turnover in HIV-infected patients. *Proc Natl Acad Sci U S A*. 2000;97:13778-13783.
- Hazenber MD, Stuart JW, Otto SA, et al. T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). *Blood*. 2000;95:249-255.
- Mohri H, Perelson AS, Tung K, et al. Increased

- turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. *J Exp Med*. 2001;194:1277-1287.
11. Kovacs JA, Lempicki RA, Sidorov IA, et al. Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV. *J Exp Med*. 2001;194:1731-1741.
 12. Anthony KB, Yoder C, Metcalf JA, 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;33:125-133.
 13. De Paoli P, Zanussi S, Simonelli C, et al. Effects of subcutaneous interleukin-2 therapy on CD4 subsets and in vitro cytokine production in HIV+ subjects. *J Clin Invest*. 1997;100:2737-2743.
 14. Levy Y, Capitant C, Houhou S, et al. Comparison of subcutaneous and intravenous interleukin-2 in asymptomatic HIV-1 infection: a randomised controlled trial. ANRS 048 study group. *Lancet*. 1999;353:1923-1929.
 15. Giorgi JV, Hultin LE, McKeating JA, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis*. 1999;179:859-870.
 16. Giorgi JV, Liu Z, Hultin LE, Cumberland WG, Hennessey K, Detels R. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr*. 1993;6:904-912.
 17. Grossman Z, Paul WE. The impact of HIV on naive T-cell homeostasis. *Nat Med*. 2000;6:976-977.
 18. Roederer M, Dubs JG, Anderson MT, Raju PA, Herzenberg LA. CD8 naive T cell counts decrease progressively in HIV-infected adults. *J Clin Invest*. 1995;95:2061-2066.
 19. Messele T, Abdulkadir M, Fontanet AL, et al. Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. *Clin Exp Immunol*. 1999;115:443-450.
 20. Kalinkovich A, Weisman Z, Greenberg Z, et al. Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. *Clin Exp Immunol*. 1998;114:414-421.
 21. Silvestri G, Sodora DL, Koup RA, et al. Non-pathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. *Immunity*. 2003;18:441-452.
 22. Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RM. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol*. 2002;169:3400-3406.
 23. Hazenberg MD, Otto SA, van Benthem BH, et al. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS*. 2003;17:1881-1888.
 24. Deeks SG, Hoh R, Grant RM, et al. CD4+ T cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy. *J Infect Dis*. 2002;185:315-323.
 25. Wallis RS, Kalayjian R, Jacobson JM, et al. A study of the immunology, virology, and safety of prednisone in HIV-1-infected subjects with CD4 cell counts of 200 to 700 mm⁻³. *J Acquir Immune Defic Syndr*. 2003;32:281-286.
 26. Calabrese LH, Lederman MM, Spritzler J, et al. Placebo-controlled trial of cyclosporin-A in HIV-1 disease: implications for solid organ transplantation. *J Acquir Immune Defic Syndr*. 2002;29:356-362.
 27. Chapuis AG, Paolo Rizzardi G, D'Agostino C, et al. Effects of mycophenolic acid on human immunodeficiency virus infection in vitro and in vivo. *Nat Med*. 2000;6:762-768.
 28. Miller KD, Masur H, Jones EC, et al. High prevalence of osteonecrosis of the femoral head in HIV-infected adults. *Ann Intern Med*. 2002;137:17-25.
 29. Abrams DI, Bebchuk JD, Denning ET, et al. Randomized, open-label study of the impact of two doses of subcutaneous recombinant interleukin-2 on viral burden in patients with HIV-1 infection and CD4+ cell counts of > or = 300/mm³: CP-CRA 059. *J Acquir Immune Defic Syndr*. 2002;29:221-231.
 30. Stellbrink HJ, van Lunzen J, Westby M, et al. Effects of interleukin-2 plus highly active antiretroviral therapy on HIV-1 replication and proviral DNA (COSMIC trial). *AIDS*. 2002;16:1479-1487.
 31. Youle M, Fisher M, Nelson M, et al. Randomised study of intermittent subcutaneous interleukin-2 (IL-2) therapy without antiretrovirals versus no treatment [abstract]. XIII International AIDS Conference, Durban, South Africa, 2000.
 32. Sereti I, Herpin B, Metcalf JA, et al. CD4 T cell expansions are associated with increased apoptosis rates of T lymphocytes during IL-2 cycles in HIV infected patients. *AIDS*. 2001;15:1765-1775.
 33. Kovacs JA, Imamichi H, Vogel S, et al. Effects of intermittent interleukin-2 therapy on plasma and tissue human immunodeficiency virus levels and quasi-species expression. *J Infect Dis*. 2000;182:1063-1069.
 34. Tavel JA, Sereti I, Walker RE, et al. A randomized, double-blinded, placebo-controlled trial of intermittent administration of interleukin-2 and prednisone in subjects infected with human immunodeficiency virus. *J Infect Dis*. 2003;188:531-536.
 35. Hengel RL, Thaker V, Pavlick MV, et al. Cutting edge: L-selectin (CD62L) expression distinguishes small resting memory CD4+ T cells that preferentially respond to recall antigen. *J Immunol*. 2003;170:28-32.
 36. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;401:708-712.
 37. Emery S, Abrams DI, Cooper DA, et al. The evaluation of subcutaneous proleukin (interleukin-2) in a randomized international trial: rationale, design, and methods of ESPRIT. *Control Clin Trials*. 2002;23:198-220.