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Rational interleukin 2 therapy for HIV positive individuals: Daily low doses enhance immune function without toxicity

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ABSTRACT **When administered in high doses to HIV positive (HIV$^+$) individuals, interleukin 2 (IL-2) causes extreme toxicity and markedly increases plasma HIV levels. Integration of the information from the structure-activity relationships of the IL-2 receptor interaction, the cellular distribution of the different classes of IL-2 receptors, and the pharmacokinetics of IL-2 provides for the rationale that low IL-2 doses should circumvent toxicity. Therefore, to identify a nontoxic, but effective and safe IL-2 treatment regimen that does not stimulate viral replication, doses of IL-2 from 62,500 to 250,000 IU/m^2/day were administered subcutaneously for 6 months to 16 HIV$^+$ individuals with 200–500 CD4+ T cells/mm^3. IL-2 was already detectable in the plasma of most HIV$^+$ individuals even before therapy. Peak plasma IL-2 levels were near saturating for high affinity IL-2 receptors in 10 individuals who received the maximum nontoxic dose, which ranged from 187,500 to 250,000 IU/m^2/day. During the 6 months of treatment at this dose range, plasma levels of proinflammatory cytokines remained undetectable, and plasma HIV RNA levels did not change significantly. However, delayed type hypersensitivity responses to common recall antigens were markedly augmented, and there were IL-2 dose-dependent increases in circulating Natural Killer cells, eosinophils, monocytes, and CD4+ T cells. Expanded clinical trials of low dose IL-2 are now warranted, especially in combination with effective antivirals to test for the prevention of immunodeficiency and the emergence of drug-resistant mutants and for the eradication of residual virions.**

Augmentation of host immunoreactivity has remained an elusive therapeutic goal despite almost a century of effort, primarily because of the potential to generate toxicity by overstimulation. However, the HIV epidemic now provides the impetus and clinical research opportunity to explore new approaches to immunotherapy. Interleukin 2 (IL-2), one of the first cytokines to be discovered and characterized, is particularly attractive as an immunotherapeutic agent, in that a great deal of basic (1) and clinical data (2, 3) concerning the activity and use of IL-2 have accumulated in the past decade. IL-2 was first used clinically for the treatment of cancer (4, 5), and initially very high IL-2 doses were employed, up to 150×10^6 IU/day, resulting in extremely toxic side effects. The toxicities limited the duration of IL-2 therapy to only a few days and restricted its use to patients hospitalized in the intensive care unit. Recently, approximately 10-fold lower doses of IL-2 have been administered to HIV positive (HIV$^+$) individuals (6). Given intermittently as i.v. infusions for 5 days every 2 months, these doses of IL-2 still cause severe toxic side effects, including capillary leak with hypotension, high fever (>39°C), and extreme flu-like symptoms, necessitating the safety of hospitalization. Moreover, detectable plasma HIV concentrations increase with each treatment course, as much as 50-fold (6).

Detailed *in vitro* experiments have shown that IL-2 promotes its effects by binding to specific receptors [IL-2 receptor (IL-2R)] (7, 8). Three distinct classes of IL-2Rs have been identified that differ by their binding affinity for IL-2. High affinity IL-2Rs, expressed by antigen-activated T cells and B cells, and a minor subset of Natural Killer (NK) cells (10%), bind IL-2 at very low concentrations, in the range of 1–100 pM. Intermediate affinity IL-2Rs, which are expressed constitutively by most NK cells, require 100-fold higher IL-2 concentrations for binding, from 100 pM to 10 nM. Low affinity IL-2Rs are not expressed on the cell surface, but they have been detected in soluble form and require very high IL-2 concentrations for binding (10–1000 nM). High doses of IL-2, which lead to systemic IL-2 concentrations that bind most NK cell intermediate affinity IL-2 receptors, stimulate the simultaneous secretion of large quantities of proinflammatory cytokines (2, 3). These NK cell-derived cytokines, including tumor necrosis factor α, interferon γ, and granulocyte-monocyte colony stimulating factor, have been implicated in the generation of the toxic inflammatory side effects of high dose IL-2 (2, 3, 9, 10). These same proinflammatory cytokines promote the *in vitro* expression of latent HIV proviral DNA (11–15), making administration of IL-2 in high doses potentially dangerous.

Although we now have a fairly complete understanding of the structure-activity relationships of IL-2Rs, this information has not yet been integrated with pharmacokinetic data to establish a therapeutic index for IL-2. *In vitro* data suggest that the IL-2 therapeutic to toxic ratio may be determined ultimately by the absolute number of IL-2R + cells stimulated. If so, treatment with ultralow doses of IL-2 should result in systemic IL-2 concentrations sufficient to saturate only the high affinity IL-2Rs, i.e. below 100 pM. This should avoid activation of most NK cells, thereby circumventing the release of large amounts of proinflammatory cytokines.

To test the hypothesis that nontoxic but therapeutic IL-2 doses could be identified and to determine whether IL-2 could be given safely to asymptomatic HIV$^+$ individuals without promoting viral replication, we patterned our approach after that described by Ritz and coworkers (16–18), who first reported that low doses of IL-2 could be given to cancer patients for periods up to 3 months with only minimal toxicity. Our results indicate that ultralow IL-2 doses are not only nontoxic and safe but also effective in stimulating immune reactivity. Accordingly, it is now feasible to consider IL-2 immunotherapy as an adjuvant to antiviral chemotherapy.

METHODS

Individuals were eligible if they were HIV antibody positive, had no concurrent opportunistic infections, had an absolute CD4+ T cell count between 200 and 500 cells/mm^3, and had been taking nucleoside-analogue antiretroviral medication for

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Abbreviations: IL-2, interleukin 2; HIV$^+$, HIV positive; NK, natural killer; bDNA, branched chain DNA assay; DTH, delayed-type hypersensitivity.

at least 1 month prior to starting IL-2 therapy. The study was approved by the institutional review board of the New York Hospital-Cornell Medical Center, and all subjects provided written informed consent after the risks associated with participation in the study had been explained to them.

Recombinant human IL-2 [R-Met-HU IL-2 (Ala-125)] was supplied by Amgen (Thousand Oaks, CA). All dosages for the study were calculated as IU, using a specific activity of 15×10^6 IU per mg of protein.

Dose-limiting toxicity (DLT) was defined as World Health Organization grade I toxicity (19). If dose-limiting toxicity occurred, treatment was withheld until the toxic symptoms or signs resolved, and treatment was then resumed at a dose midway between the previous dose level and the current dose.

Plasma IL-2, tumor necrosis factor α, interferon γ, and granulocyte-monocyte colony stimulating factor concentrations were determined by ELISA (Endogen, Cambridge, MA) according to the manufacturer's specifications. Determination of leukocyte types and concentrations were performed by three-color flow cytometry (20). Plasma HIV levels were determined by branched chain DNA assay (bDNA) (21, 22).

Recall antigens for *Candida albicans* (Miles), dermatophyton (Miles), trichophyton (Miles), and mumps virus (Connaught Laboratories, Swiftwater, PA) were used to test for delayed-type hypersensitivity (DTH) prior to IL-2 therapy and at the termination of the 6-month study interval. Induration $\geq$5 mm in diameter was considered positive.

For statistical analysis, categorical variables were evaluated by a Fisher exact test. Linear trends in cell counts and branched chain DNA assay over time were calculated by computing linear regression slopes separately for each patient, and then the average slopes for each dose level were compared using the Student's *t*-test. For eosinophils, 1 month values were compared with baseline by the paired Student's *t*-test.

RESULTS

Patient Characteristics. Sixteen individuals were enrolled in the study (Table 1). The mean age of the subjects was 37 years (range 28–48), and the mean time of known HIV infection was 7 years. Nine individuals had been taking antiretroviral nucleoside analogue medication for at least 6 months. Three subjects (subjects 6, 12, and 16) began antinucleoside medication 6 weeks prior to initiation of IL-2 therapy. The mean CD4+ T cell count prior to entrance on study was 347 $\pm$ 24 (SEM). The group was homogeneous with respect to leukocyte and differential counts.

Dose Modifications and Side Effects. The first four individuals (subjects 1–4) initially received 500,000 IU/m^2, but all developed systemic symptoms within a few days, so that their doses were reduced to 125,000 IU/m^2/day. Two of these individuals (subjects 1 and 2) had medical histories of mild allergic asthma and experienced a worsening of asthma symptoms that correlated with each IL-2 injection. Cessation of IL-2 therapy resulted in attenuation of symptoms. Consequently the IL-2 dose was reduced by 50%, to 62,500 IU/m^2/day, and these individuals completed the study without any other untoward effects, as did the next four individuals (subjects 3–6) who received 125,000 IU/m^2/day.

Ten individuals (subjects 7–16) were enrolled at 250,000 IU/m^2/day. At this dose, three subjects (subjects 7, 8, and 13) experienced fever to 38.5°C, myalgia and fatigue, with the onset of systemic symptoms occurring after 4 -5 days, and 6–8 h after each IL-2 injection. Accordingly, the dose was decreased to 187,500 IU/m^2/day, which was tolerated throughout 6 months of therapy without further side effects. The remaining seven individuals completed 6 months of therapy at 250,000 IU/m^2/day without significant systemic symptoms. Therefore, the maximum dose range tolerated for 6 months without systemic side effects or significant laboratory abnormalities was 187,500 IU to 250,000 IU/m^2/day.

IL-2 Pharmacokinetics. To determine the relationship between IL-2 dose and the systemic IL-2 concentrations attained, plasma IL-2 levels were determined before and at frequent intervals for 24 h after each IL-2 dose increment. Unexpectedly, low but detectable levels of IL-2 were found in 11 of 16 plasma samples even before therapy. The IL-2 concentrations detected ranged from 0.5 to 9 pM (Fig. 1*A*). By comparison, plasma samples from 10 normals were all $<$0.5 pM, the lower limit of detection for the assay.

As shown in Fig. 1*B*, at each IL-2 dose level the peak plasma IL-2 concentration occurred at 2 h, and as the IL-2 dose was increased, the peak plasma IL-2 level increased concomitantly. Most noteworthy, 125,000 IU/m^2 led to only half-saturating peak plasma IL-2 concentrations, and these concentrations were dissipated rapidly, returning to baseline after 8 h. At the maximum nontoxic dose, 250,000 IU/m^2, the peak plasma IL-2 concentration was near saturating, 22 pM, and at 500,000 IU the peak concentration was even higher, 35 pM. Measurement of trough IL-2 levels 24 h after each maximum nontoxic dose

Table 1. Study subject characteristics

Subject	Age	Year of diagnosis	Antiviral (duration in months)	Cells/mm^3					
				CD4+ T Cell	WBC	Lymphs	Monos	PMNs	Eos
1	44	1987	ddC (3)	280	5200	1872	374	2704	213
2	28	1991	AZT (12)	495	5600	1982	672	2800	84
3	48	1987	AZT (84), ddC (24)	263	3400	1020	374	1938	68
4	42	1985	AZT (2)	270	9000	2340	270	6120	117
5	48	1983	AZT (24)	382	4800	1152	288	3216	77
6	32	1989	AZT (1.5)	307	5600	1909	560	2520	56
7	34	1987	AZT (6)	240	5100	1377	612	3009	87
8	35	1984	d4T (12)	476	4400	1892	484	1892	97
9	33	1994	AZT (12), ddC (2)	316	5300	1219	636	3339	58
10	42	1986	AZT (6), d4T (6)	282	5900	2313	625	2773	159
11	35	1990	3TC (8). d4T (3)	202	4700	1410	470	2773	71
12	28	1993	ddI (1.5)	438	5700	1653	399	3249	342
13	32	1989	d4T (6)	306	6900	2346	690	3381	414
14	38	1986	ddI (4), d4T (1)	473	4400	1408	440	2376	88
15	37	1990	AZT (50), ddI (50)	462	7200	1030	828	5054	209
16	41	1989	d4T (1.5)	356	5600	1753	515	3013	230
Mean	37	1988		347	5550	1667	515	3135	148
SEM	2	1		24	326	114	39	268	27

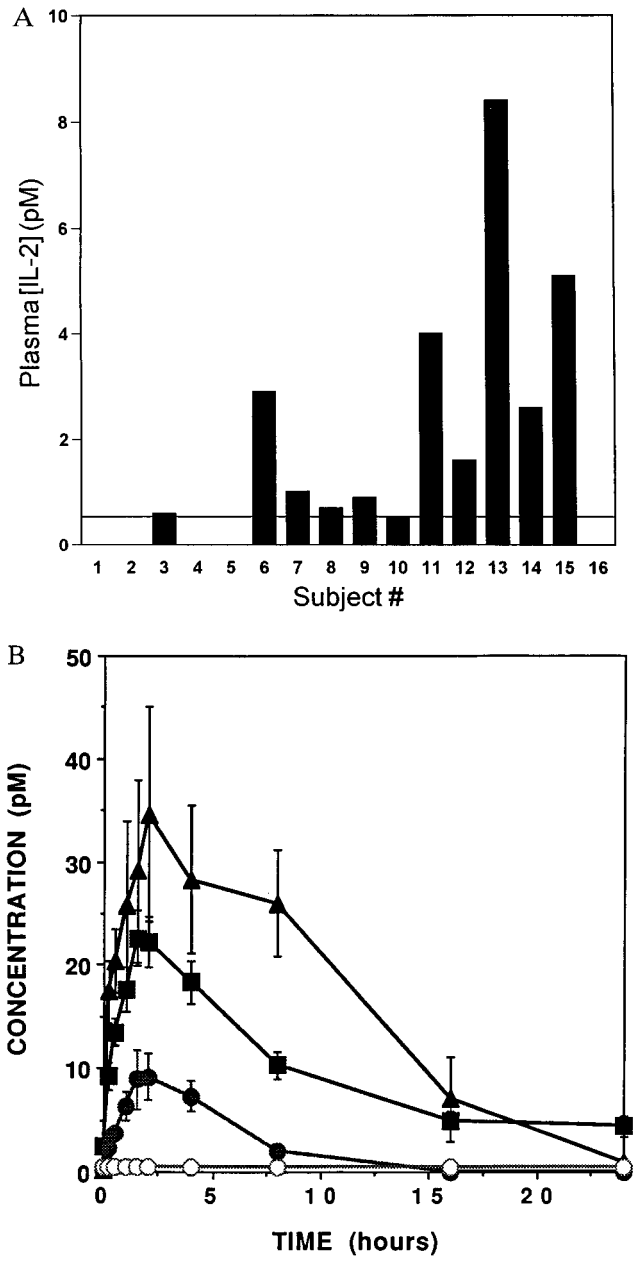

FIG. 1. Plasma cytokine concentrations. (*A*) Determinations of plasma IL-2 concentration on samples obtained prior to IL-2 therapy from the 16 study subjects. The horizontal line at 0.5 pM delimits the lower limit of detection of the assay. Samples from 10 normal individuals were all below this limit. (*B*) Mean (± SEM) determinations of plasma IL-2 concentrations on samples from 6 subjects who received 125,000 IU/m^2 (●), 10 subjects who received 250,000 IU/m^2 (■), and 4 subjects who received 500,000 IU/m^2 (▲), assayed at intervals over 24 h. The open symbols represent negative test results for plasma tumor necrosis factor α, interferon γ, and granulocyte-monocyte colony stimulating factor in the 10 subjects who received the maximum nontoxic dose of 250,000 IU/m^2.

revealed that further daily doses of IL-2 did not exceed clearance, so that gradually increasing plasma IL-2 levels did not occur. In addition, at the maximum nontoxic dose, plasma levels of the proinflammatory cytokines, tumor necrosis factor α, interferon γ, and granulocyte-monocyte colony stimulating factor remained below the limits of detection throughout the 6-month treatment interval.

Plasma HIV Concentration. The first six subjects, who received ≤125,00 IU/m^2/day, were monitored for plasma HIV RNA levels with a bDNA that had a sensitivity of 10,000 viral RNA molecules/ml (21). Prior to IL-2 therapy, only two of these subjects (subjects 2 and 6) had detectable plasma HIV (14 and 20 × 10^3 viral RNA molecules/ml); the assays of these two individuals became negative after the initiation of IL-2 treatment and remained so throughout the 6 months of the study. Subsequently, a second generation bDNA assay with a lower limit of detection of 500 HIV RNA molecules/ml became available. With this improved bDNA assay, the 10 individuals who received the maximum, nontoxic IL-2 dose range (subjects 7–16) had a mean plasma HIV concentration of 8547 ± 4764 (SEM) RNA molecules/ml at the initiation of IL-2 therapy (Table 2). Over the course of IL-2 therapy, monthly determinations of the mean HIV concentration fluctuated <2 fold, and at the end of the study the HIV titer was not significantly different from that at the beginning, 7753 ± 2081 (SEM) ($P > 0.1$).

DTH Responses. DTH reactions test for T cell memory in response to the intradermal injection of common environmental antigens such as *C. albicans*, trichophyton, and dermatophyton or to vaccines such as mumps virus and tetanus toxoid. Therefore, DTH tests were used to assess antigen-specific cell-mediated immunity before and at the end of 6 months of IL-2 treatment. The DTH test results for the group that received ≤125,000 IU/m^2/day revealed that three of the six individuals converted their test for *C. albicans* from negative to positive after 6 months of IL-2 administration, while tests for mumps and trichophyton remained negative. In the group that received the maximal nontoxic dose range, four of the 10 individuals converted at least one test from negative to positive after 6 months of IL-2 administration. Most noteworthy, in this group the mean diameters of the DTH tests were >2-fold greater after IL-2 therapy than before therapy (Fig. 2) ($P < 0.005$).

White Blood Cells. There were no significant changes in the absolute neutrophil counts, CD8+ T cell counts, or B cell counts during the 6 months of therapy from the values obtained prior to IL-2 therapy in either the group that received ≤125,000 IU/m^2/day or the group that received the maximum nontoxic dose. In contrast, as shown in Fig. 3*A*, there was almost a 6-fold increase in the mean concentration of circulating NK cells in the group that received the maximal nontoxic dose after 6 months of treatment, compared with an insignificant change from baseline NK cells in the group that received ≤125,000 IU/m^2/day ($P = 0.04$). The concentrations of circulating eosinophils also changed, but unlike the progressive increase of NK cells, eosinophils increased after only 2 weeks of IL-2 inoculations, peaked at 1 month ($P = 0.01$), and remained elevated 2-fold above baseline beyond 2 months (Fig. 3*B*). Also, as shown in Fig. 3*C*, at the lower IL-2 doses the monocyte counts decreased slightly during the study interval, whereas at the maximal nontoxic dose range there was an increase in circulating monocytes that peaked after 2 months of IL-2 treatment, and remained elevated 2–3-fold from baseline throughout the remainder of the study ($P = 0.04$).

At the initiation of the study, the concentration of CD4+ T cells for all 16 subjects was 383 ± 45 cells/mm^3 (mean ± SE). During 6 months of IL-2 therapy at IL-2 doses ≤125,000 IU/m^2/day, there was a progressive decline in CD4+ T cells (to 276 ± 52/mm^3), while at the maximal nontoxic dose range, a progressive increase in CD4+ T cells occurred, reaching 543 ± 110/mm^3 at 6 months, for a mean monthly gain of 27 cells/mm^3 (Fig. 3*D*). To utilize all of the monthly data obtained for each subject, the slopes of the changes in the CD4+ T cell concentrations were determined for those receiving the maximum nontoxic dose range and those receiving ≤125,000 IU/m^2. Linear trends were then calculated from the mean of these slopes. From this analysis, there was a mean monthly gain in CD4+ T cells of 28 cells/mm^3 (95% CI = −2, 58) in the group receiving the maximal nontoxic dose range, whereas there was a mean monthly loss of 28 cells/mm^3 (95% confi-

Table 2. Plasma HIV concentration (RNA Molecules/ml).

Subject	0*	1*	2*	4*	8*	12*	16*	20*	24*
7	2,832	3,919	3,748	2,894	4,605	2,795	1,140	1,296	2,815
8	3,190	4,792	4,765	1,941	11,070	8,916	12,430	1,910	10,440
9	546	2,049	789	ND*	ND	ND	ND	ND	ND
10	1,910	1,260	—	ND	4,585	3,719	1,070	3,958	3,387
11	2,209	9,702	6,950	4,875	4,785	1,296	7,523	7,662	9,110
12	22,090	27,150	13,300	13,430	32,810	28,790	18,380	20,200	21,130
13	1,868	5,949	4,978	6,331	9,565	24,330	4,864	15,550	5,554
14	779	ND	622	ND	ND	ND	ND	ND	811
15	2,650	2,481	2,065	1,563	15,220	7,577	7,217	9,063	13,980
16	47,400	54,850	50,680	76,170	61,900	36,140	47,710	53,020	10,300
Mean	8,547	11,215	9,766	10,720	14,454	11,356	10,033	11,266	7,753
SEM	4,764	5,444	5,273	7,387	6,083	4,213	4,591	5,114	2,081

*Duration of IL-2 therapy (weeks).
†ND = not detectable.

dence interval = −49, −7) in the group that received ≤125,000 IU/m^2/day (P = 0.01).

DISCUSSION

The results from this study indicate that asymptomatic HIV$^+$ individuals can self-administer IL-2 safely and without any detectable toxicity for 6 months. This is the first report of chronic daily administration of low dose IL-2 for such a prolonged period. The maximum nontoxic doses range from 187,500 to 250,000 IU/m^2/day. At these doses, plasma HIV RNA levels did not change significantly from baseline, yet there was readily detectable stimulation of the immune system, as evidenced by augmentation of DTH responses and by increases in circulating NK cells, eosinophils, monocytes, and CD4+ T cells. The dose-limiting toxicities included weakness, fatigue, lethargy, myalgias, and low grade fever. These symptoms occurred within a few days after beginning the IL-2 injections but could be circumvented by lowering the daily IL-2 dose. There were no toxicities to the major organ systems detected by laboratory assays throughout 6 months of daily therapy. Accordingly, subjects were able to self-administer IL-2 and perform normal daily activities without hindrance related to IL-2 side effects.

Finding low levels of IL-2 in the plasma of HIV$^+$ individuals prior to therapy was unanticipated, particularly as these individuals were asymptomatic, presenting with no systemic symptoms indicative of an ongoing immunologic reaction, such as fever or fatigue. Circulating IL-2 is undetectable in normal individuals and has only been reported thus far in the plasma of patients undergoing severe immunologic reactions, such as renal transplant rejection (23). Formerly, IL-2 was thought to be a cytokine that functioned only in the microenvironment of a site of inflammation or within a lymph node (1). However, as IL-2 can now be detected in the plasma of most asymptomatic HIV$^+$ individuals, the administration of IL-2 can be seen as an augmentation of a normal systemic immunologic reaction already in progress. In this regard, the detectable IL-2 plasma levels are entirely consistent with the recent findings of the rapid turnover of plasma virus and circulating CD4+ T cells (24).

The peak plasma IL-2 concentration of 22 pM attained at the maximum nontoxic dose of 250,000 IU/m^2 is sufficient to saturate ~70% of high affinity IL-2Rs, expressed by activated T cells and B cells and by a minor subset of NK cells (7, 8, 25–27). In contrast, this concentration would be expected to bind only 2% of the intermediate affinity IL-2R expressed by most NK cells. Accordingly, the 100-fold difference in the equilibrium dissociation constants of these IL-2Rs (i.e. K_d = 10

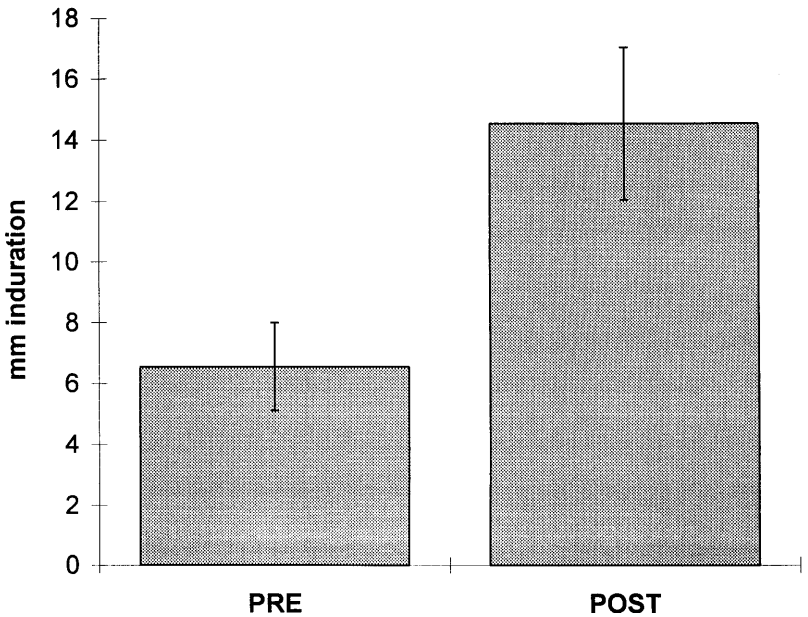

FIG. 2. Mean diameters (± SEM) of the positive delayed type hypersensitivity tests performed on the 10 subjects who received the maximum tolerated doses (187,500–250,000 IU/m^2/day) for 6 months. Pretherapy, 14 of 30 tests were positive, and posttherapy 18 of 30 tests were positive.

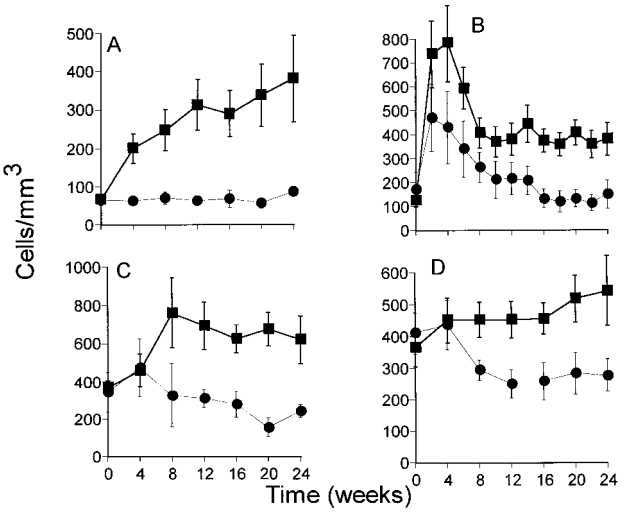

FIG. 3. Circulating white blood cell concentrations at monthly intervals during IL-2 therapy at 125,000 IU/m^2/day (mean ± SE, 6 subjects) (●), and at the maximum nontoxic doses (187,000–250,000 IU/m^2/day) (mean ± SEM, 10 subjects) (■). (*A*) NK cells; (*B*) eosinophils; (*C*) monocytes; (*D*) CD4+ T cells.

versus 1000 pM) provides the therapeutic index. Until this study, systemic toxicity was considered unavoidable. However, our findings indicate that as long as the IL-2 dose is kept low enough to lead to plasma concentrations that only saturate high affinity IL-2Rs, there is no toxicity. In this regard, it is noteworthy that the therapeutic range is narrow, in that peak plasma IL-2 concentrations that exceed 30 pM lead to toxic side effects.

As predictable by the lack of toxic symptoms, at the maximum nontoxic dose IL-2 also does not trigger the burst in viral production reported previously for high dose IV therapy (6). For example, after 6–18 million IU administered IV, there was a marked increase in plasma HIV, that ranged as high as 50-fold and persisted for several weeks beyond the 5-day infusion (6, 22). This increase in virus production is likely due to the stimulatory effect of secondary proinflammatory cytokines, which are also responsible for the toxic symptoms observed with high dose IL-2 administration. By comparison, the 50-fold lower doses of IL-2 given as daily subcutaneous inoculations in the present study did not result in systemic toxicity nor in a significant change in plasma viral levels from the values obtained pretreatment.

The identification of a nontoxic dose of IL-2 is only significant if this dose is shown to be therapeutic. Therefore, the IL-2 dose-dependent increases in the number of circulating NK cells, eosinophils, monocytes, and CD4+ T cells are especially noteworthy. No other reported therapy for HIV$^+$ individuals has been shown to potentiate the circulating concentration of all of these cells, while also augmenting DTH to common recall antigens. Of the various types of cells that increased during 6 months of therapy at the maximum nontoxic dose, the NK cells are likely to be critical to the overall response. NK cells are important for host defenses to a wide variety of viral infections, including Epstein–Barr virus, cytomegalovirus, herpes simplex, and herpes zoster virus (28). Moreover, in a recent report of 347 HIV$^+$ patients, NK cell activity was found to be suppressed compared with 110 normals (29). *In vitro*, NK cells respond to IL-2 by an increased cytotoxic capability and by secreting cytokines that are potent stimuli for monocytes and macrophages (1, 25, 26). *In vivo*, it is likely that the IL-2-promoted accumulation of circulating NK cells results in an enhanced cytolytic capacity, and the IL-2-promoted release of the secondary cytokines in small quantities should recruit and activate monocytes and macrophages, which play prominent roles in innate immune responses. Also, the augmentation of antigen processing and presentation by monocytes and macrophages, promoted particularly by NK cell-derived cytokines, would be expected to augment antigen-specific, acquired immunoreactivity. The increased number of circulating monocytes observed at the maximum nontoxic dose should also contribute to improved host defenses. Therefore, continuous stimulation of NK cells by daily low doses of IL-2 can augment the NK cell-monocyte interaction without generating the toxicity observed following high dose intermittent IL-2 administration.

An increase in circulating CD4+ T cells was unexpected, as earlier trials using low dose IL-2 for a shorter duration (1–3 months) failed to result in a significant change in CD4+ T cells, whether the underlying disease was cancer (16–18), HIV infection (30), or AIDS-associated malignancies (31). It is noteworthy that the rate of increase in CD4+ T cells of 27–28 cells/mm^3/month is relatively rapid compared with the experience with antivirals. For example, there was a reported increase of only four cells/mm^3/month during the first 6 months of zidovudine therapy (32), of 10 cells/mm^3/month during 6 months of combination therapy with zidovudine and lamivudine (3TC) (33), and of ~20 cells/mm^3/month during 6 months of triple therapy with zidovudine, lamividune, and the protease inhibitor, indinavir (34).

The mechanism responsible for the increase in CD4+ T cells observed with low dose IL-2 therapy is likely to be different from that promoted by antiviral therapy. Since the IL-2 therapy did not change the mean plasma HIV RNA concentration, the increase in circulating CD4+ T cells is more likely due to an acceleration of T cell production, or less likely, to a redistribution of cells from the lymphoid organs to the circulation. In contrast, the increase in CD4+ T cells that occurs with antiretroviral chemotherapy has been attributed to a decrease in the rate of CD4+ T cell destruction (24, 35, 36). Further studies will be required to determine whether the IL-2-promoted gain in CD4+ T cells is attributable to an enhanced rate of maturation of CD4+ T cell precursors versus the proliferative expansion of mature peripheral T cells or to a combination of these mechanisms. However, it is important that the increase in circulating CD4+ T cells was reflected in improved functional cell mediated immune responses as monitored by DTH tests. Therefore, finding that IL-2 therapy augments not only the number of CD4+ T cells but also increases NK cells and monocytes and enhances the functional capability of cell mediated immune responses to environmental antigens offers the hope that IL-2 immunotherapy may well prevent or retard the appearance of opportunistic infections.

Recent studies detailing viral clearance rate, infected CD4+ T cell life span, and viral generation time, have revealed that there are at least two identifiable viral compartments (24). The vast majority of the circulating virions (i.e. ≥99%) are derived from productively infected CD4+ T cells that are destined to die rapidly ($t_{1/2}$ = 1.6 days). By comparison <1% of plasma virions can be attributed to a residual viral pool in latently infected CD4+ T cells and long-lived tissue macrophages. It is now hopeful that antivirals may markedly reduce the rapidly replicating viral pool in the productively infected CD4+ T cells. However, such therapy may not be effective in combating the residual viral pool (24). Accordingly, IL-2 immunotherapy may well be necessary as an adjuvant to antiviral chemotherapy to completely suppress virus production.

On the basis of our results, a trial is now indicated to determine whether daily low dose IL-2 therapy beyond 6 months will continue to potentiate immune reactivity and delay the onset of AIDS. In addition, because low dose IL-2 does not stimulate HIV replication, the augmentation of host defenses promoted by IL-2 should synergize with the decrease in immune system destruction promoted by antiviral agents, so that additional trials combining IL-2 immunotherapy and

antiretroviral chemotherapy appear warranted. In particular, combined therapy of individuals earlier in the course of HIV infection (i.e. when CD4+ T cells $\geq$500 cells/mm^3) should now be examined.

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