

Prolonged Immunostimulatory Effect of Low-Dose Polyethylene Glycol Interleukin 2 in Patients with Human Immunodeficiency Virus Type 1 Infection

By Hedy Teppler,* Gilla Kaplan,* Kendall A. Smith,†
Antonia L. Montana,* Peter Meyn,* and Zanvil A. Cohn*

From the *Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021; and †Immunology Research, Dartmouth Medical School, Hanover, New Hampshire 03755

Summary

13 patients with human immunodeficiency virus type 1 infection class II–IV, but without opportunistic infection or neoplasm, received 6 μg (3.6×10^4 IU) of polyethylene glycol recombinant human interleukin 2 (PEG IL-2) intradermally twice a week for 4 mo and were then followed for an additional 6 mo. Clinical, immunological, and viral parameters were monitored in the patients, all of whom were taking zidovudine. The cutaneous administration of PEG IL-2 resulted in an indurated zone resembling a delayed-type hypersensitivity response of 26 ± 1 -mm diameter (676 mm^2) at 72–96 h after injection throughout the 4 mo of administration. This dose, which was appreciably lower than in most previous trials, was not associated with local or systemic toxicity. No increase in the viral burden of circulating leukocytes or plasma occurred. A number of immunological functions were stimulated by this course of therapy. All patients demonstrated high levels of lymphokine-activated killer cell activity by cells freshly removed from the circulation and in the absence of in vitro exposure to IL-2. Natural killer cell activity was also enhanced. Limiting dilution analysis revealed an increase in the frequency of IL-2-responsive cells from abnormally low to levels above normal during the course of injections. In a subgroup of four patients with ≥ 400 CD4^+ T cells/ μl at entry, there was a trend to sustained increases in CD4^+ T cell numbers. However, this increase did not reach statistical significance. This subset of patients also exhibited higher proliferative responses to phytohemagglutinin as mitogen. Several of these effects persisted for 3–6 mo after cessation of therapy. In conclusion, low-dose IL-2 regimens lead to sustained immune enhancement in the absence of toxicity. We suggest pursuit of this approach for further clinical trials both as prophylaxis and therapy.

The loss of CD4^+ T cells during the course of HIV-1 infection is accompanied by a reduction in IL-2 (1), a proximally situated lymphokine required for both cellular and humoral immunity (2). As a result, life-threatening opportunistic infections and neoplasms ultimately supervene (3, 4). Although antiretroviral agents and specific prophylactic agents have slowed progression of opportunistic infection, the morbidity and mortality rates remain high (5, 6).

It has been shown that in vitro addition of IL-2 to cells from HIV-1-infected patients can correct a number of their immunological abnormalities, enhancing antigen and mitogen responsiveness and NK cell function (7–12). Based on these findings there was interest in treating HIV-1-infected patients

with IL-2 in vivo. Early clinical trials used IL-2 from a variety of biological sources and at varied dosing regimens. There was short-term improvement in some parameters of immune function such as delayed-type hypersensitivity (DTH)¹ and CD4^+ T cell count, but no overall reconstitution of the immune system (13–18). In these early trials, patients had advanced HIV disease, mostly AIDS, and antiretroviral therapy was not yet available. There was dose-dependent toxicity with IL-2 treatment, as had been seen in cancer trials using IL-2 (19).

Recombinant human IL-2 (rhIL-2) is now available and is of high biological activity (20). We have demonstrated that rhIL-2 given in much lower doses by the intradermal route can modify local and systemic immunity in lepromatous

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¹ Abbreviations used in this paper: DTH, delayed-type hypersensitivity; PEG, polyethylene glycol.

leprosy (21, 22). This is associated with a body-wide infiltration into the skin of CD4⁺ and CD8⁺ T cells, monocytes, and Langerhans cells, and results in a significant reduction in the mycobacterial burden without IL-2 toxicity.

More recently, we have reported that a single low dose (10 µg; 180,000 IU) of rhIL-2 given to HIV-1-positive patients can reconstitute their DTH skin test reactions when given at a site distant from that of IL-2 administration (23). In addition, in a 1-mo trial with 10 µg rhIL-2 (180,000 IU) given daily by the intradermal route, both local cell emigration in response to IL-2 and systemic immune responses were enhanced, and these changes were evoked in the absence of toxicity (24, 25). The use of polyethylene glycol rhIL-2 (PEG IL-2),¹ which has a plasma clearance 15-fold lower than unmodified rhIL-2, allowed administration only twice a week (23, 24, and PEG IL-2 Investigator's brochure, Cetus Oncology, Chiron Corp., June 1, 1990). A dose of 6 µg (36,000 IU) resulted in larger DTH reactions than unmodified rhIL-2 and increased NK cell and LAK cytotoxicity without IL-2 toxicity (25). In these multiple-dose studies, patients were required to take zidovudine because of the possibility that IL-2 would increase viral replication in T cells. In light of the evidence that low-dose PEG IL-2 could be given safely and led to enhancement of immune function, we initiated a 4-mo study that will now be reported.

Materials and Methods

Patients. 16 HIV-1-seropositive patients were recruited from a cohort of patients followed at The Rockefeller University Hospital in a longitudinal study (26), or were self-referred after learning of our trial from friends or through the protocol listings in the AIDS Treatment Registry in New York City and in the National Institutes of Health AIDS database. Patients were included in the study if they met the following criteria: CD4⁺ T cell count of $\geq 100 \mu\text{l}$ (one patient had CD4⁺ T cell count of $64/\mu\text{l}$ at entry), no active opportunistic infection or neoplasm requiring systemic chemotherapy, and no other immunomodulatory drugs within 12 wk of study entry. All patients were required to take zidovudine, at least 300 mg daily, because of the possible risk of enhanced HIV-1 replication. The protocol was approved by the Institutional Review Board of The Rockefeller University Hospital, and all patients gave written informed consent. Patients were seen in the outpatient unit of The Rockefeller University Hospital.

Study Design. At baseline evaluation, all patients underwent history and physical examination. Laboratory evaluation included complete blood count, differential, and routine biochemical profile. CD4⁺, CD8⁺, and CD3⁺ T cells were enumerated. DTH responses to control antigens were assessed using *Candida albicans* (Connaught Laboratories, Inc., Swiftwater, PA) and tetanus toxoid (aluminum phosphate adsorbed; Wyeth Laboratories, Inc., Marietta, PA), 0.1 ml each, by intradermal injection to the forearm. DTH responses to HIV-1 antigens were measured using the HIV-1 immunogen skin test, an envelope-depleted, killed virus preparation (a generous gift of Immunization Products, Ltd., Horsham, PA), 10 µg in 0.1 ml by intradermal injection. All DTH responses were read at 48–72 h. The size of the response was expressed as the product of the two greatest diameters (in mm²). Selected DTH sites were biopsied using a 4-mm punch. Immunohistochemistry was performed on these sections as previously described (25).

After enrollment, patients received 36,000 IU PEG IL-2 (Cetus

Oncology Division, Chiron Corporation, Emeryville, CA) twice weekly on a Monday/Thursday or Tuesday/Friday schedule for up to 4 mo at the outpatient unit of The Rockefeller University Hospital. The drug was given by intradermal injection to rotating sites in the skin of the back. Patients were queried for evidence of toxicity and examined at each visit. Clinical laboratory parameters as well as selected in vitro analyses were repeated at 5 wk, 2 mo, and 4 mo of PEG IL-2 therapy, and in follow-up at 3 and 6 mo. Skin tests were repeated at 5 wk, 2 mo, and 4 months of therapy, and 3 mo after completion of therapy.

PEG IL-2. rhIL-2 is produced by *Escherichia coli* into which the cDNA coding for human IL-2 has been cloned. It has similar physical and biological properties compared to native IL-2 (20). rhIL-2 (Cetus Oncology, Chiron Corp.) has a specific activity of 18×10^6 IU/mg. PEG IL-2 is a modified form of rhIL-2 in which the polymer is covalently attached to rhIL-2. It has a plasma clearance 10-fold lower than unmodified rhIL-2 (PEG IL-2 Investigator's brochure, Cetus Oncology, Chiron Corp., June 1, 1990). The specific activity of PEG IL-2 used in this study was 6×10^6 IU/mg.

In Vitro Analysis. Approximately 60–75 ml of peripheral blood was collected in heparinized tubes. After removal of the supernatant plasma, PBMC were obtained by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ), and resuspended in RPMI 1640, 2 mM glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin (Gibco Laboratories, Grand Island, NY) with 10% normal human serum (Biocell, Cardon, CA) as previously described (25). Serum and plasma were prepared in a standard manner.

Cytotoxicity Assays. These were performed using a ⁵¹Cr (New England Nuclear, Boston, MA) release assay at various E/T ratios in triplicate as previously described (25). NK cell activity was determined using ⁵¹Cr-labeled K562 cell targets, and LAK (27) cell activity assays used ⁵¹Cr-labeled Daudi cell targets. Effector cells were either fresh PBMC on the day of isolation (NK and LAK assays) or were cells that had been cultivated in vitro for 5 d with or without rhIL-2 at 600 IU/ml (3.3 ng/ml) (LAK assays). Results are expressed as percent specific ⁵¹Cr release as calculated from the following formula: $100 \times [(\text{mean cpm experimental} - \text{mean cpm spontaneous release}) / (\text{mean cpm total release} - \text{mean cpm spontaneous release})]$. Values shown are mean \pm SEM for all patient samples at a specified E/T ratio, and at the time point indicated.

Limiting Dilution Analysis. The frequency of circulating IL-2-responsive cells was determined using a limiting dilution analysis of PBMC after the method of Lefkowitz (28). In a 96-well plate, 5×10^4 irradiated feeder cells (patient PBMC) were added per well, and fresh PBMC were added at 2×10^4 followed by a series of twofold dilutions in the presence of 28,000 IU/ml (155 ng/ml, or 10 nM) rhIL-2. After 10 d, cells were pulsed with [³H]thymidine (ICN Radiochemicals, Irvine, CA). 18 h later, cells were harvested, and [³H]thymidine uptake was measured. Responder wells were defined as those with [³H]thymidine uptake of greater than the mean plus three times the SD for cells with media alone. The frequency of responsive cells was then converted to a percentage for further statistical analysis. PBMC from normal laboratory volunteers were analyzed in the same manner.

Lymphocyte Transformation Tests. These assays were performed using phytohemagglutinin (PHA-P; Difco Laboratories, Detroit, MI) as mitogen in a 3-d [³H]thymidine uptake assay as previously described (25). The killed HIV-1 immunogen was also tested for its ability to stimulate lymphocyte proliferation at the following concentrations: 5.0, 0.5, and 0.05 µg/well, or 25, 2.5, and 0.25 µg/ml in a 7-d assay.

Flow Cytometry. The phenotype of peripheral PBMC was analyzed by flow cytometry as previously described (25), and used the following fluorochrome-conjugated antibodies: Leu-4 (CD3), Leu-3a (CD4), Leu-2a (CD8), HLA-DR (MHC class II), Leu-11c (CD16), the low affinity IL-2R (anti-Tac, CD25), Leu-M3 (CD14) (all from Becton Dickinson & Co., Mountain View, CA), and NKH-1 (CD56) (Coulter Immunology, Hialeah, FL). For all antibodies except Leu-M3, the population analyzed was gated by scatter criteria as the lymphocyte population. For Leu-M3 analysis, total PBMC (lymphocytes plus monocytes) were used.

Viral Culture. HIV-1 burden in PBMC as well as plasma was determined in a coculture system with normal lymphoblasts using HIV p24 ELISA (DuPont Co., Wilmington, DE) as the endpoint. This was done in bulk and in 10-fold dilutions for end-point analysis as previously described (25) in a method adapted from Ho et al. (29) and Coombs et al. (30). Viral load was determined by time to positive bulk culture as well as the tissue culture infectious dose by endpoint dilution.

Statistical Analysis. Results of all in vitro analyses are expressed as the mean \pm SEM. CD4⁺ T cell counts and limiting dilution analysis of the frequency of IL-2-responsive cells were analyzed by Mann-Whitney U-Wilcoxon rank sum *W* test. Results of cytotoxicity assays were tested by analysis of variance, comparing values of samples receiving identical in vitro treatment (cells analyzed on the day of isolation; cells cultivated with or without IL-2 in vitro) from baseline to PEG IL-2 treatment and follow-up timepoints.

Results

Patient Characteristics and Clinical Course

16 patients, 26–65 yr old, Center for Disease Control class II–IV (31, 32), were enrolled in the study (Tables 1 and 2).

Table 1. Patient Characteristics at Enrollment

Patient	Age	Sex	CDC class	Diagnosis	Entry CD4 ⁺ T cells	Medications
					<i>per μl</i>	
1	36	M	IVC.2	OHL	364	ZDV, ACV
2	36	M	II	ASX	304	ZDV, ACV, AP
3	61	M	II	ASX	360	ZDV
4	33	M	IVC.2	Thrush	210	ZDV, ACV, TMP-SMZ
5	26	M	II	ASX	459	ZDV
6	37	M	IVC.2	Thrush	122	ZDV, ACV, TMP-SMZ
7	38	M	IVC.2	OHL	309	ZDV, ACV
8	65	M	II	ASX	443	ZDV
9	28	F	II	ASX	499	ZDV
10	37	M	IVC.2	Thrush	300	ZDV, TMP-SMZ
11	29	M	II	ASX	414	ZDV
12	27	M	II	ASX	352	ZDV
13	41	F	III	PGL	441	ZDV
14	57	M	II	ASX	120	ZDV, ACV, DAPSONE
15	39	M	IVA	ARC	494	ZDV
16	34	M	IVC.2	OHL	64	ZDV, TMP-SMZ

Diagnosis: ASX, asymptomatic; PGL, persistent generalized lymphadenopathy; Thrush, oral candidiasis; OHL, oral hairy leukoplakia; ARC, AIDS-related complex. Medications: ZDV, zidovudine; ACV, acyclovir; AP, aerosol pentamidine; TMP-SMZ, trimethoprim-sulfamethoxazole.

There were 14 men and 2 women. Five patients (nos. 1, 2, 3, 5, and 10) had received rhIL-2 or PEG IL-2 in prior studies at least 12 wk before study entry. In the current study 13 patients received at least eight injections (21 ± 2 injections per patient, mean \pm SEM) over a 4-mo period and were included for analysis. Of the three patients who received less than eight injections, one (no. 15) dropped out immediately and one (no. 6) 3 wk after enrollment. One patient (no. 10) developed a fever of unknown origin and was taken off zidovudine by his private physician after 3 wk on study. He was therefore ineligible to receive further PEG IL-2.

Of the 13 patients included in the analysis, 10 completed at least 12 wk of treatment. Of the three patients who received <12 wk of PEG IL-2, patient no. 14 developed grade 1 (mild) anemia and his zidovudine was stopped by his personal physician at 8 wk of study. He therefore could not receive further PEG IL-2. Patient no. 16, whose entry CD4⁺ T cell count was $64/\mu$ l, developed mild *Pneumocystis carinii* pneumonia at week 8 of the study and after a brief hospitalization completed his treatment as an outpatient. The third patient (no. 13) entered the study at the midpoint but received all doses from the time of entry until the completion of the study.

Adverse Events

Of the 13 initial patients, two (nos. 9 and 11) reported a mild flu-like illness with headache, low grade fever, and myalgias for 1–2 d over the 4-mo treatment period. Patient no. 5 had intermittent, transient, and asymptomatic hypo-

Table 2. Patient Characteristics at Enrollment and Number of Doses PEG IL-2

Patient	(>4 DTH mm)		Prior IL-2	Total no. of doses/total wk
	Candida	Tetanus		
1	-	+	Y	27/16
2	+	+	Y	27/16
3	-	+	Y	26/16
4	+	+	N	9/12
5	-	+	Y	23/16
6	-	+	N	5/3
7	+	+	N	21/12
8	+	-*	N	32/16
9	+	+	N	28/16
10	+	+	Y	5/3
11	+	+	N	24/16
12	+	+	N	25/15
13	+	+	N	12/6
14	-	+	N	12/8
15	ND	ND	N	0/0
16	-	-	N	11/16

* This patient had a late positive DTH response to tetanus toxoid (see text).

glycemia (glucose, 40–45 mg/dl) over the period of observation, including at the 6-mo posttreatment visit. As noted above, patient no. 14 developed grade 1 anemia. None of these symptoms or signs could be clearly related to the administration of PEG IL-2.

Local Response

At the site of injection we observed an area of induration and erythema clinically similar to a DTH response (21, 23, 25). The average size of the reaction was 26 ± 1 mm in diameter (mean \pm SEM) at 72–96 h after injection, and this area of 676-mm² did not vary appreciably during the course of PEG IL-2 injections. The injections were well tolerated, with only mild pruritus at the injection sites.

Hematologic Responses

During the course of PEG IL-2 treatment, there was an upward trend of the CD4⁺ T cell count, which did not reach statistical significance. When patients were stratified into groups based on entry CD4⁺ T cell counts per microliter of ≥ 400 or < 400 (Fig. 1), there was a more pronounced increase in CD4⁺ T cells in the group of patients with entry counts $\geq 400/\mu\text{l}$. This increase was 20–39% above baseline during treatment and at 3 mo of follow-up. By 6 mo of follow-up the CD4⁺ T cell count declined to 13% above baseline. However, the number of patients in this subset

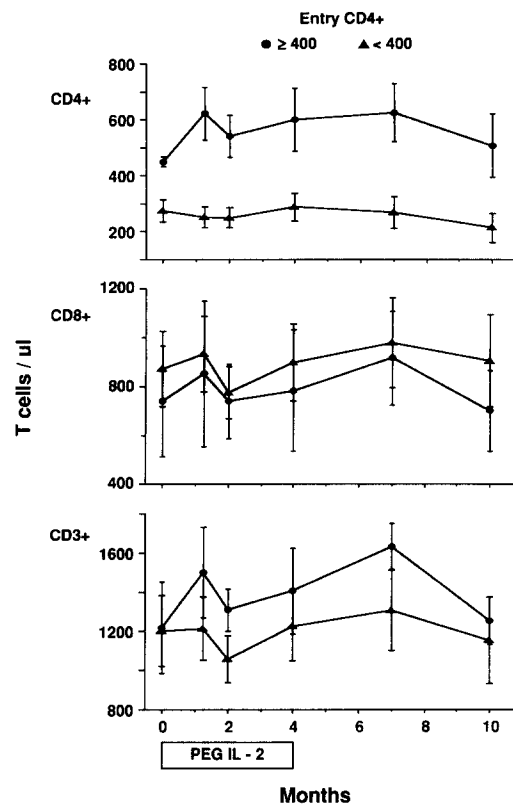


Figure 1. T cell profile over the course of PEG IL-2 treatment and follow-up. Patients with entry CD4⁺ T cells $\geq 400/\mu\text{l}$ (●, $n = 4$), or $< 400/\mu\text{l}$ (▲, $n = 7$). Values shown are mean \pm SEM.

was too small to demonstrate statistical significance by Wilcoxon rank sum analysis. In the group with entry CD4⁺ T cell numbers $< 400/\mu\text{l}$, CD4⁺ T cell counts were maintained but did not increase. The overall or stratified CD8⁺ and CD3⁺ T cell counts did not change significantly from baseline over the course of PEG IL-2 treatment. Overall total white blood cell count and lymphocyte counts were also unchanged.

Cytotoxicity

LAK Cell Activity. LAK cell activity is assayed by the ability of PBMC to kill Daudi cell targets. Cells from our patients at baseline, as well as from normal controls, failed to show significant LAK activity when freshly isolated or when cultivated in the absence of IL-2. Both normal and patient populations expressed high activity after 5 d of cultivation in the presence of 600 IU/ml IL-2.

Significant alterations in LAK cell activity were apparent at 2 and 4 mo of PEG IL-2 administration. During treatment, freshly isolated PBMC exhibited markedly increased LAK activity without in vitro cultivation ($p = 0.001$ at 4 mo of treatment; Fig. 2 A). In fact, PBMC cultivated for 5 d in the absence of IL-2 still demonstrated maximal ability to kill Daudi targets ($p < 0.001$ at 2 mo of treatment; Fig. 2 B). These effects persisted at 3 and 6 mo of follow-up ($p < 0.05$ for fresh PBMC and $p < 0.001$ for cultivated cells). It

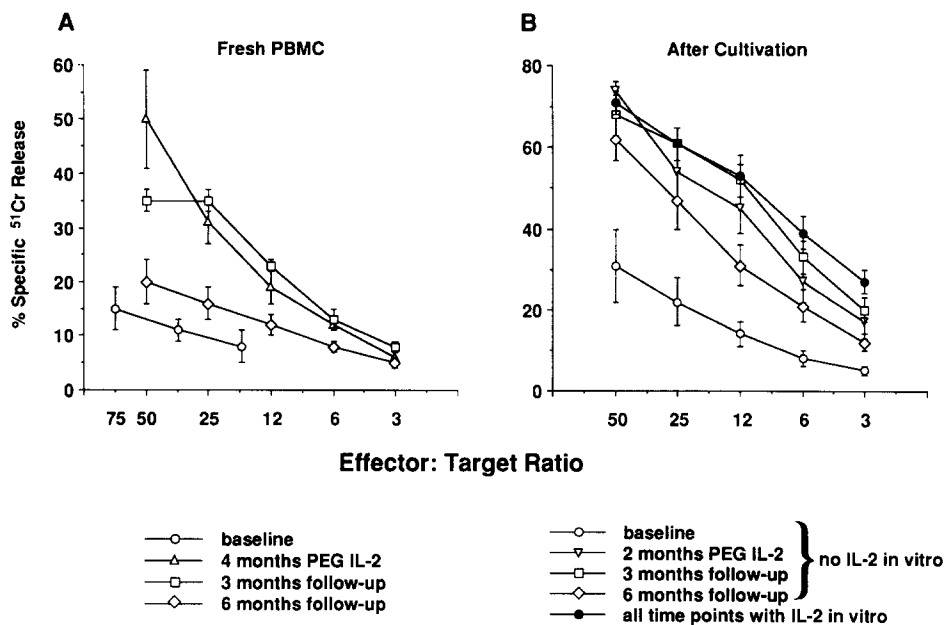


Figure 2. Enhanced LAK cell activity during PEG IL-2 treatment and follow-up. Patient PBMC were assayed (A) fresh on the day of isolation (mean, $n = 7$) or (B) after 5 d of cultivation with (mean, $n = 9$) or without (mean, $n = 8$) rhIL-2 (600 IU/ml or 3.3 ng/ml) in vitro. (Open symbols) Cells that have received no rhIL-2 in vitro. (●) Mean values for PBMC stimulated by IL-2 in vitro for all patients at all time points. Values shown are mean \pm SEM.

therefore appeared that PEG IL-2 enhanced LAK cell activity in vivo.

NK Cell Activity. We examined the ability of fresh PBMC to kill the K562 cell, a NK cell target (Fig. 3). Enhancement of NK cell activity was evident at 2 mo ($p = 0.001$), 4 mo (data not shown), and persisted at 3 mo of follow-up ($p < 0.001$). By 6 mo of follow-up, this enhancement was no longer seen.

Frequency of IL-2-responsive Cells

Limiting dilution analysis was performed to assess the percentage of IL-2-responsive cells in the circulation as determined by proliferative responses (Fig. 4). In normal controls ($n = 7$) the percentage of IL-2-responsive cells among PBMC

is $1.88 \pm 0.66\%$ (mean \pm SEM). In our patients at baseline the percentage of IL-2-responsive PBMC was reduced more than fourfold to $0.42 \pm 0.08\%$. This increased at 2 and 4 mo of treatment, and at 4 mo, the percentage of IL-2-responsive cells was $4.88 \pm 1.81\%$, an increase of >2.5 -fold above the value for controls, and >10 -fold above patient baseline values ($p = 0.0008$). This effect subsided completely by 3 mo of follow-up. The phenotype of the IL-2-responsive population has not as yet been ascertained.

Lymphocyte Proliferative Responses

The ability of PBMC to proliferate in response to mitogen (PHA) or HIV-1 antigen was examined. After PEG IL-2 administration and through 3 mo of follow-up, there was a 1.5-

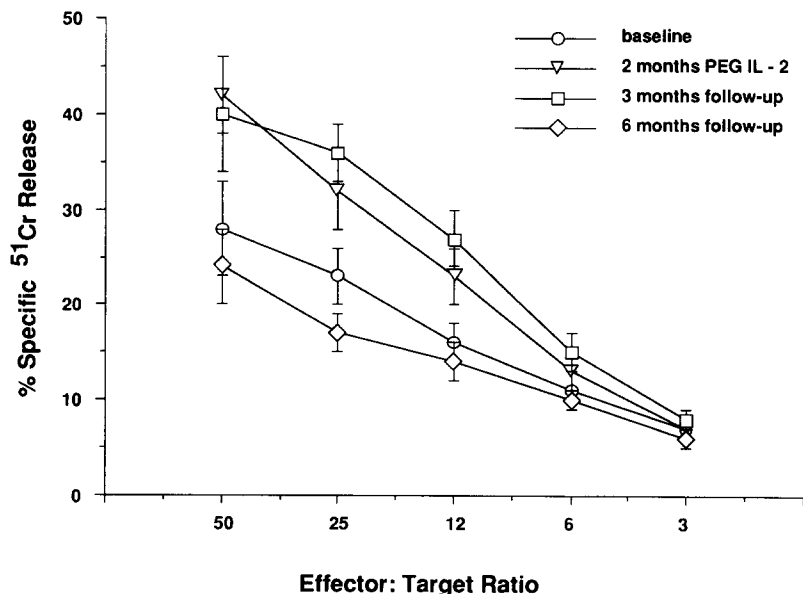


Figure 3. Enhanced NK cell activity over the course of PEG IL-2 treatment and follow-up (mean, $n = 10$). Values shown are mean \pm SEM.

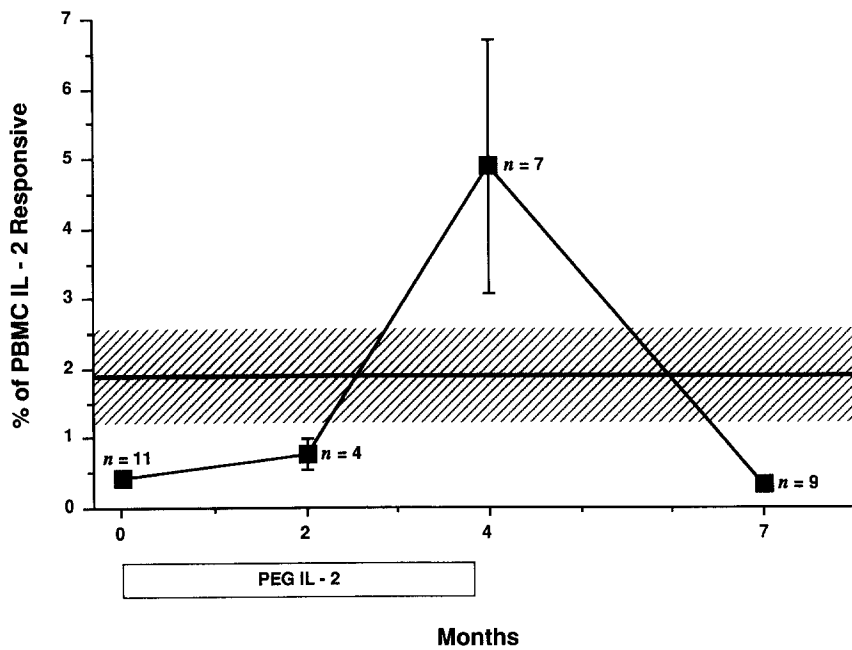


Figure 4. Percentage of PBMC that are IL-2 responsive. Frequency of IL-2-responsive cells among PBMC was determined by limiting dilution analysis, and converted into percentages. Values shown are the mean \pm SEM for all patients tested at the time point indicated. Value for controls is shown as hatched area representing mean \pm SEM. Open bar indicates duration of PEG IL-2 treatment.

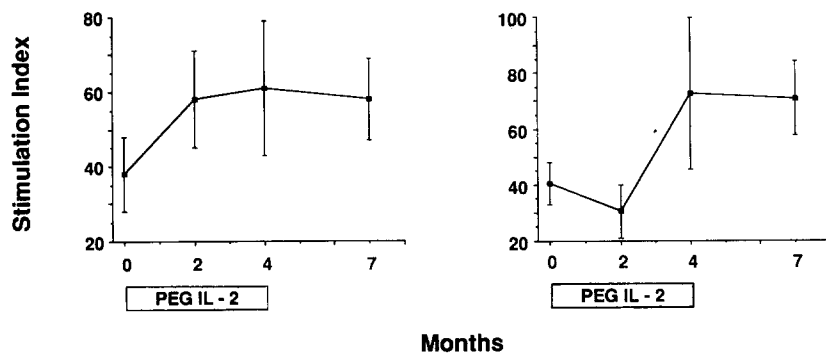


Figure 5. Lymphoproliferative responses to PHA during PEG IL-2 treatment and follow-up. Lymphocyte proliferative responses to PHA during treatment and follow-up in all patients (*left*) and in patients with entry CD4⁺ T cell counts $\geq 400/\mu\text{l}$ (*right*). Stimulation index equals mean cpm stimulated cells/mean cpm cells in media alone. Values shown are mean \pm SEM. Duration of PEG IL-2 administration indicated by open bars.

fold increase in proliferative response to PHA, but this did not reach statistical significance (Fig. 5, *left*). In the group of patients with entry CD4⁺ T cell counts $\geq 400/\mu\text{l}$, however, this increase was significant (Fig. 5, *right*) and was sustained at the 3-mo follow-up evaluation.

Proliferative responses to the HIV-1 antigen in vitro were quite low at all time points, but did increase fivefold in all patients and threefold in the group with entry CD4⁺ T cell counts $\geq 400/\mu\text{l}$ (Table 3). At follow-up these values returned to baseline. These responses were only observed at the higher antigen dose level of 25 $\mu\text{g}/\text{ml}$.

PBMC Phenotype. A number of other parameters were followed and were essentially unchanged. These included the phenotype of peripheral PBMC as determined by immunofluorescence and flow cytometry, other than in the CD4⁺ T cells noted earlier. There was no significant change in the gated lymphocyte population in total T cells (CD3⁺), NK cells (CD56⁺), class II MHC-expressing cells (HLA-DR⁺), or in cells that express the low affinity IL-2 receptor, p55 (CD25). There was also no change in monocyte numbers (CD14⁺) (data not shown). This was true for the entire

group of patients as well as for the stratified groups based on entry CD4 T cell counts.

Quantitation of HIV-1 Burden. There was no consistent or sustained change in the titer of cell-associated virus or plasma

Table 3. Proliferative Responses to HIV-1 Antigen

Timepoint	All patients	Entry CD4 ⁺ T cells ($\geq 400/\mu\text{l}$)
Baseline	0.5 \pm 0.1	0.5 \pm 0.2
2 mo PEG IL-2	1.2 \pm 0.3	1.2 \pm 0.1
4 mo PEG IL-2	2.4 \pm 0.7	1.5 \pm 0.3
3 mo follow-up	0.9 \pm 0.4	0.6 \pm 0.4

Values shown are mean \pm SEM of stimulation indices at 25 $\mu\text{g}/\text{ml}$ of antigen. Stimulation index is mean cpm stimulated cells/mean cpm cells in media alone.

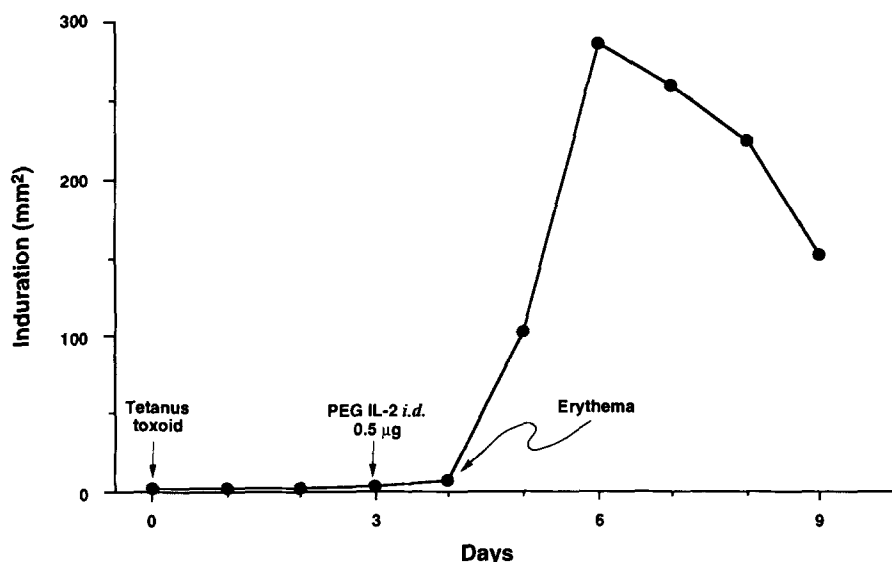


Figure 6. Effect of PEG IL-2 on the DTH response to tetanus toxoid. This patient was anergic to intradermal tetanus toxoid 2 d after injection. On day 3 a single dose of 0.5 μg or 2,000 IU PEG IL-2 was given at a distant site. Within 24 h there was erythema at the forearm tetanus toxoid site, and by 48–72 h after receiving PEG IL-2, the tetanus site became indurated. This response waned over the next few days.

viremia during PEG IL-2 treatment and follow-up (data not shown). All patients had virus isolated from PBMC at entry (TCID, 10^4 to 5×10^6) and three patients had negative PBMC viral cultures at one time point during therapy, but follow-up cultures showed PBMC titers comparable to those at baseline. There was low titer plasma viremia (TCID, 1.0 and 0.1 ml) in up to three patients at any time point.

DTH Responses

The enhancement in the size of DTH reactions by rhIL-2 and PEG IL-2 has been commented upon in previous publications (21, 23, 25). Examples of enhancement of response to tetanus toxoid were noted in this study, including one late positive tetanus toxoid DTH response. This patient (no. 8) was initially anergic at 48 h to tetanus toxoid administered to the volar forearm, and his first dose of PEG IL-2 was administered to the back at that visit. He then demonstrated a typical DTH response at the tetanus site that peaked at 3 d after his dose of PEG IL-2. The late positive tetanus toxoid DTH response is shown in Fig. 6, in a patient who participated in a 1-mo PEG IL-2 trial (25). His originally anergic tetanus site became strongly positive only after his first and lowest dose (0.5 μg or 2,000 IU) of PEG IL-2 was given in the dose escalation phase of the study.

The HIV skin test was performed in 12 of 13 patients. Only one (no. 10) had a positive, 4-mm diameter response before PEG IL-2. Late positive HIV skin tests were observed after the first dose of PEG IL-2 in three additional patients. This occurred only in patients after punch biopsy of what were considered negative sites at 48–72 h after placement of the HIV skin test. In these individuals, 10–20-mm-diameter areas of erythema and induration occurred around the biopsy site. At 5 wk, 2 and 4 mo, and at 3 mo of follow-up, the HIV skin tests were uniformly negative.

Discussion

The use of low-dose PEG IL-2 (36,000 IU) given twice weekly by intradermal injection has resulted in sustained enhancement of a number of immune parameters. This has occurred without any apparent toxicity related to IL-2 and without evidence of any change in viral burden. Two indices of systemic immunity were particularly enhanced. First, the percentage of PBMC that proliferate in response to IL-2, in a limiting dilution assay, was increased 12-fold. The percentage of IL-2-responsive cells returned to baseline upon withdrawal of the lymphokine. Second, there were striking increases in the activity of LAK cells, and to a lesser degree in the activity of NK cells. Cells removed from the circulation exhibited high levels of LAK cytotoxic activity without further *in vitro* incubation with IL-2. In fact, incubation with IL-2 for 5 d failed to enhance activity above that expressed by cells that had been incubated without IL-2. Of interest was the persistence of increased LAK and NK activity many months beyond the cessation of therapy, suggesting a long-lived population. Both of these parameters of immunity were enhanced in all patients irrespective of their original CD4⁺ T cell counts.

There were, however, IL-2-induced effects that appeared to require a critical number of preexisting CD4⁺ T cells. This was most evident in the ability of IL-2 to increase and maintain CD4⁺ T cells in patients with entry CD4⁺ T cell counts of $\geq 400/\mu\text{l}$ during the 4-mo trial. Due to the small number of patients in this subgroup, this change was not statistically significant. In contrast, patients with < 400 CD4⁺ T cells per microliter at the outset failed to exhibit increased numbers, but maintained their original levels. Similarly, the enhanced proliferative responses to the mitogen, PHA, were most apparent in patients with ≥ 400 CD4⁺ T cells per microliter at entry. In studies with Dr. M. J. McElrath

(University of Washington, Seattle, WA), we have noted that patients with CD4⁺ T cell counts of <50/μl fail to generate maximal indurative responses to intradermal PEG IL-2 itself, nor can their DTH reactions be enhanced. Yet, during PEG IL-2 treatment, three of six patients in this series cleared or improved their chronic oral candidiasis (our unpublished data).

It should be noted that all patients were taking zidovudine (ZDV) during PEG IL-2 administration. ZDV may have blunted the cellular response to the lymphokine. Our experience with the HIV-1-infected patients differed from that with leprosy patients (22) and from the experience of others treating patients with cancer and after bone marrow transplantation with low to moderate doses of IL-2 for prolonged periods (33–37). Patients with lepromatous leprosy who received low-dose (10 μg or 180,000 IU BID) intradermal rhIL-2 developed both lymphadenopathy in the regional nodes of the axilla (injection sites were in the upper back), and brisk elevations in the number of CD4⁺ and NKH-1⁺ (NK) lymphocytes in the circulation, which were not seen in the HIV-1-infected population. Alternatively, the dose of PEG IL-2 may have been too low to cause an increase in the number of NK cells, since data from cancer chemotherapy and bone marrow transplantation trials show significant expansion of NK cell numbers, and in the cancer trials (35) this expansion was IL-2 dose dependent.

In a recent trial of rhIL-2 in patients with early HIV infection, CD4⁺ T cells ≥400/μl and no prior antiretroviral therapy, a relatively high dose of IL-2 (1.5–12 × 10⁶ IU/m²/d, or 83–667 μg/m²/d) was given by 24-h continuous intravenous infusion 5 d/wk for 1 mo plus ZDV with the expected IL-2 toxicities (38). Transient increases in CD4⁺ T cell numbers were seen during therapy. Interestingly, many of these patients showed sustained elevations of CD4⁺ T cells for up to 18 mo of follow-up, but as these patients were newly started on antiretroviral therapy the relative contributions of IL-2 and ZDV in this rise are not clear. During IL-2 treatment, enhanced NK and LAK activity were demonstrated, but only in the toxic, high-dose range (6–12 × 10⁶ IU or 333–667 μg/m²/d). In addition there was a de-

crease in HIV-1 provirus in PBMC as determined by semi-quantitative PCR (39). In contrast to this study, we have demonstrated significantly enhanced NK and LAK activity at nontoxic doses of IL-2. These increases were much more dramatic in our patients at 2 and 4 mo of treatment than was observed in our 1-mo trial at the same doses (25). When these data are taken in aggregate with the increased frequency of IL-2-responsive cells among PBMC, and with the improved lymphoproliferative responses, both greater at 4 than 2 mo of therapy, there is a suggestion of greater cumulative effect with low-dose PEG IL-2 over a long course of therapy. Longer term trials are needed to determine the maximal immune enhancement achievable with IL-2. This finding is supported by recent studies of low- to intermediate-dose IL-2 given by continuous infusion to patients with advanced cancer (0.5–6.0 × 10⁵ IU or 3.3–40 μg/m²/d) (34, 35) and after bone marrow transplantation (2 × 10⁵ IU or 13 μg/m²/d) (33). In these studies, expansion of the NK cell population increased progressively over a 3-mo course of IL-2 treatment, without any indication that a maximum had been reached. There was also enhancement of NK and LAK activity in vitro. There has not been evidence of enhanced LAK activity in vivo in other reports.

Further studies are needed to correlate the enhanced immunological parameters with the incidence and severity of opportunistic infections. Based upon our present results and data from animal experiments (40), we suspect that long-term IL-2 therapy represents a rational prophylactic agent that would prolong the disease-free state. The efficacy of such therapy will probably be associated with the initial levels of CD4⁺ T cells, and its use in earlier stages of HIV-1 infection is suggested. We consider that therapy can be administered easily by the patients themselves at home by the subcutaneous route. This could be on a twice weekly schedule with PEG IL-2 or daily with rhIL-2. With both preparations, the persisting local indurative response would be an excellent indication of compliance. Such studies are planned for the near future.

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Address correspondence to Hedy Tepler, Division of Infectious Diseases, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107.

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