The Systemic Influence of Recombinant Interleukin 2 on the Manifestations of Lepromatous Leprosy

By Gilla Kaplan,* Warwick J. Britton,‡ Gerald E. Hancock,* Willem J. Theuvenet,‡ Kendall A. Smith,§ C. K. Job,‖ Paul W. Roche,‡ Anthony Molloy,* Rochel Burkhardt,* Jeanne Barker,* Hira M. Pradhan,¶ and Zanvil A. Cohn*

*From *The Rockefeller University, New York, New York 10021; ‡The Leprosy Mission Anandaban Hospital, Kathmandu, Nepal; §Dartmouth Medical School, Hanover, New Hampshire 03756; ‖Gillis W. Long Hansen's Disease Center, Carville, Louisiana 70721; and the ¶Leprosy control section, Division of Public Health, Teku, Kathmandu, Nepal*

Summary

14 patients with lepromatous leprosy received twice daily injections of 10 μg recombinant interleukin 2 (rIL-2), by the intradermal route, in the skin of the back for 8 d (total dose, 160 μg). Lymphokine administration was accomplished without drug toxicity, or the development of acute nerve damage. The majority of patients developed nontender axillary lymphadenopathy during the course of treatment. Local injection sites showed progressively larger zones of induration, peaking at 24 h and persisting for many days. Early 12-h reactions were of a macular, erythematous nature and exhibited an increasingly striking diurnal variation. The morning injection sites were three- to fourfold larger in diameter than those placed in the evening (9 am to 9 pm). Systemic manifestations of intradermal rIL-2 administration were noted. Peripheral blood T cells, including $CD4^+$ and $CD8^+$ phenotypes, increased 2–2.5-fold and NK cells increased sixfold. Elevations in [^{3}H]TdR incorporation into peripheral blood mononuclear cells occurred to a variety of mycobacterial antigens, but not to those of *Mycobacterium leprae*. Within 2 wk, biopsies at sites far removed from the back showed increased infiltration of mononuclear cells in 12 of 14 patients. Immunocytochemistry revealed the presence of newly emigrated $CD4^+$ T cells, monocytes, and dermal $CD1^+$ Langerhans cells. Endothelial cells of small dermal vessels expressed major histocompatibility complex class II determinants on their surface. Transmission electron microscopy of these specimens revealed markedly enlarged endothelial cells with many surface projections extending into the lumen as well as extravasating lymphoid cells. The numbers of acid-fast *M. leprae* in the peripheral sites were examined by slit smear and in biopsies of matched leprosy lesions taken before and after IL-2 administration. Within 2 mo, slit smears showed a 0.5 log or greater reduction in 12 of 14 patients, with a mean for all patients tested of 0.5 log units. Biopsy specimens showed a 1 log unit or greater reduction in the bacterial index (B.I.) in 6 of 14 patients. Historical controls in this Nepalese population showed a 0.5 log unit reduction after multidrug therapy over a period of 12 mo. Thus, after 8 d of IL-2 injections, a fivefold reduction in B.I. was observed during the first 2 mo of the study. Antibody levels against *M. leprae* phenolic glycolipid 1 (PGL-1) and lipoarabinomanan B were markedly elevated after IL-2 injections, while PGL-1 antigen levels were reduced. We conclude that the administration of rIL-2 has had a significant effect in decreasing the total body burden of *M. leprae*. This is accomplished by the influx of mononuclear leukocytes from the circulation, the enhancement of cell-mediated immunity, and the degradation of leprosy bacilli. This occurs much more rapidly compared with multidrug chemotherapy alone.

Patients with lepromatous leprosy lack detectable cellular immunity directed against *Mycobacterium leprae*. This anergy is specific, and a vigorous cell-mediated response may occur to other mycobacterial species, indicating they have all

J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/04/0993/14 $2.00
Volume 173 April 1991 993–1006

the components necessary to mount a typical delayed-type hypersensitivity (DTH)[1] reaction.

During the course of studies designed to dissect the various aspects of cell-mediated immunity, we discovered that the intradermal administration of 10–25 μg of the lymphokine IL-2 will stimulate a reaction indistinguishable from an antigen-initiated DTH response (1–4), having the hallmarks: (*a*) induration and erythema at the site; (*b*) extensive and prolonged dermal infiltration by T cells, monocytes, and Langerhans cells; (*c*) high $CD4^+/CD8^+$ T cell ratios in the emigratory population; (*d*) the absence of infiltration by granulocytic elements; (*e*) differentiation of mononuclear phagocytes to epithelioid and giant cells; (*f*) enhanced growth of overlying keratinocytes and the induction of their MHC class II antigens and IP-10 synthesis; (*g*) the generation of circulating antigen-specific and -nonspecific cytotoxic effector cells; (*h*) the destruction of parasitized macrophages; (*i*) the killing and clearance of *M. leprae*.

When administered to patients with lepromatous leprosy, a single intradermal injection of 10–25 μg rIL-2 reconstitutes the local cell-mediated reaction leading to the destruction of *M. leprae* in the cutaneous site (5). IL-2 inoculation also enhanced the responsiveness of circulating lymphocytes to antigens to which the patient had previously been sensitized (6). In addition, the level of antigen-specific T cell as well as nonselective lymphokine activated killer (LAK) cell cytotoxicity against susceptible cell line targets and human monocytes was increased (7).

These encouraging results, obtained with one to three injections in the local dermal milieu, prompted us to extend these studies to a multiple dose regimen. After a total dose of 160 μg of rIL-2, administered at 10-μg injections each 12 h for 8 d (16 injections), the systemic cell-mediated immune response of lepromatous leprosy patients is enhanced resulting in the reduction of the total body burden of leprosy bacilli.

Materials and Methods

Patient Population. 14 patients, 12–56 yr old, 10 with a clinical diagnosis of polar lepromatous leprosy (LL), two with subpolar lepromatous leprosy (SLL), and two with borderline lepromatous leprosy (BL) (8), were selected for intradermal administration of rIL-2 (Cetus Corp., Emeryville, CA) (see Table 1). Patients were included in the study if they were diagnosed as LL/BL, had a bacterial index (B.I.) (at diagnosis) by slit smear of 3+ or higher, and had received multidrug therapy (MDT) for no longer than 6 mo. Patients were hospitalized at The Anandaban Leprosy Mission Hospital, Kathmandu, Nepal. Leprosy patients in reaction or on steroid treatment were excluded from the study. Patients were informed of the nature of the study and written consent was obtained from all participants.

Patients had been treated from 1 to 6 mo, and had received MDT with 2 wk of supervised Rifampin (600 mg/d) followed by pulse therapy with Rifampin (600 mg/mo), Dapsone (100 mg/d), and clofazamine (50 mg/d and 300 mg/mo). One patient received only Dapsone (100 mg/d) for 2 yr.

After patients were admitted, routine laboratory tests were performed on blood and urine. A general clinical and neurological exam was conducted. These were repeated during and at the end of the study. All chemotherapy was continued during the trial. Slit smears taken from the same six sites including a lesion in each patient were obtained for B.I. evaluation and performed at the time of diagnosis. Patients were retested a day or two before IL-2 injection and at intervals thereafter. 30 historical controls were selected and evaluated for their rate of reduction in B.I. during 24 mo of therapy. The controls were age and sex matched, clinically matched, and had a mean ± S.D. B.I. at diagnosis of 3.6 ± 1.2 (range, 3–5+). Since all patients in the study had been treated (MDT) for at least 1 mo (mean, 4 mo), the rate of reduction in B.I. for the historical controls was calculated from 1 to 24 mo of MDT. Starting after the initial 1 or 3 mo of MDT (which includes intensive daily MDT for the first 2 wk) a mean B.I. reduction of 0.6 or 0.5, respectively, log reduction per 12 mo of therapy was observed (for linear regression analysis for the best fit for all points plotted, see Fig. 7 *inset*).

IL-2 Administration. Human rIL-2 (1.8×10^7 IU/mg protein) was transported and stored at 4°C until used. The lyophilized IL-2 was reconstituted in pyrogen-free sterile water and diluted in 5% dextrose to a concentration of 100 μg/ml, and used within 2 h of reconstitution. Patients received a dose of 10 μg rIL-2 at 12-h intervals into apparently normal skin of the back on alternate sides. Local erythema and induration of the injected site were evaluated twice daily.

Histopathology and Enumeration of Bacilli. Biopsies (wedge shaped, 1 cm in length) of matched, distal lepromatous lesions not injected with IL-2 and not in areas within the lymphatic drainage of injected sites were taken before, 5–13 d after the first IL-2 injection, and 6 mo later. The biopsies taken before and 6 mo after IL-2 injection were removed from the same lesion, and the 5–13-day biopsy was taken from another clinically matched lesion. In addition, a biopsy was taken from an area of the back not injected with IL-2 but within the lymphatic drainage of injected sites. Biopsies were cut into three parts and processed for evaluation. A part of each biopsy was fixed in 10% neutral buffered formalin overnight, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological diagnosis and with acid-fast staining for enumeration of *M. leprae*. A logarithmic index of bacilli in the biopsies (9, 10) was used to express bacterial numbers in sites obtained before, during, and after IL-2 injection.

Immunohistology. Biopsy specimens were fixed in paraformaldehyde (3%), lysine (0.075 M), and sodium-*m*-periodate (0.01 M) in PBS (PLP) for 3 h at 4°C as described (11). This fixative preserves structural details without destroying the serological reactivity of the cell surface. The biopsies were washed in PBS containing sucrose (10%) and digitonin (5×10^{-5} M) and then serially suspended in graded solutions of sucrose (15–25%). The tissue was stored in PBS with sucrose/glycerol (25% and 5%, respectively) for transport back to the U.S. until frozen.

Biopsies were embedded in OCT compound and frozen at −20°C; 6–8-μm sections were cut on a cryostat and applied to gelatin-coated multi-well slides (Carlson Scientific Inc., Peotone, IL). The sections were dried overnight at 37°C, rehydrated in PBS, and incubated with mouse mAbs followed by biotinylated horse anti-mouse Ig and then avidin-biotin peroxidase complexes (Vector Laboratories, Inc., Burlingame, CA). The reaction product was de-

[1] *Abbreviations used in this paper:* BCG, M. bovis Bacillus Calmette-Guerin; B.I., bacterial index; BL, borderline lepromatous leprosy; DTH, delayed-type hypersensitivity; LAK, lymphokine activated killer cell; LAM-B, lipoarabinomanan B; LL, lepromatous leprosy; MDT, multidrug therapy; PGL-1, phenolic glycolipid 1; PPD, purified protein derivative; SLL, subpolar lepromatous leprosy.

Table 1. *Patient Information and Clinical Status*

Patient no.	Age	Sex	B.I. at diagnosis	Clinical diagnosis‡	MDT Rx§	Day of post-IL-2 blood and biopsy
	yr				*mo*	
1	56	M	4.0	LL	6‖	13
2	35	M	4.25	LL	1	13
3	48	M	5.25	LL	6	9
4	35	M	5.0	LL	4¶	9
5	36	M	4.5	SLL	6	9
6	46	M	4.25	SLL	5	9
7	40	M	5.25	LL	6¶	5
8	45	M	3.75	BL	5	5
9	12	M	4.5	LL	1¶	5
10	33	M	4.0	LL	3¶	5
11	38	F	4.8	LL	4	5
12	55	F	4.0	LL	1	13
13	40	F	4.0	BL	6¶	13
14	45	F	3.0	LL	24**	13

* Slit smears at six sites.
‡ According to the Ridley Jopling classification, see Materials and Methods.
§ MDT in months.
‖ No. of months of pulse MDT = monthly Rifampicin (600 mg) and clofazamine (300 mg) + daily DDS (100 mg) and Clofazamine (50 mg).
¶ Intensive daily 600 μg of Rifampicin for 14 d plus MDT for numbers of months indicated.
** 24 mo of DDS (100 mg/d only).

veloped with 0.8 mg/ml 3-amino-9-ethylcarbazole and 0.015% H_2O_2. Sections were counterstained with hematoxylin.

Monoclonal and Polyclonal Antibodies for Tissue Staining. Mouse mAbs were used for the identification of specific cell types. Leu-1, Leu-2a, and Leu-3a (12, 13) (anti-CD5, -CD4, and -CD8, anti-T cells) and Leu-M5 (anti-CD11c, anti-monocyte/macrophage) (14) were obtained from Becton Dickinson & Co. (Mountain View, CA). OKT6 (15) (anti-CD1, anti-Langerhans cells) was obtained from Ortho Diagnostic Systems, Inc. (Westwood, MA). Antibody 9.3F10 (anti-MHC class II antigen) was produced in this laboratory (16). Rabbit anti-γ IP-10 antibodies were produced in this laboratory (17).

Electron Microscopy. A part of each biopsy was processed for transmission EM studies. Biopsies were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 0.1 M sucrose, pH 7.4, for 16 h at 4°C, postfixed in 2% OsO_4 for 1 h at 4°C, stained in block for 1 h with uranyl acetate in 70% ethanol, dehydrated in increments with ethanol, and embedded in epon blocks. Sections were stained with lead citrate, and examined with a transmission electron microscope (JEM 100CX; Jeol USA, Peabody, MA). At least 200 cells from each patient were examined and photographed on Kodak electron image film.

PBMC Phenotyping. Indirect immunofluorescent staining was used to identify blood mononuclear cell types and their subsets. Blood mononuclear cells (2–4 × 10^4 cells) were allowed to settle on poly-L-lysine (50 μg/ml; Sigma Chemical Co., St. Louis, MO, USA)-coated multi-well slides (Carlson Scientific Inc.) for 30–60 min on ice, quenched with 1% BSA, and incubated for 60 min with hybridoma (obtained from Dr. Robert Evans, Sloan-Kettering Memorial Hospital, New York) supernatants containing mAbs directed against CD3 (Leu-4), CD4 (Leu-3), and CD8 (Leu-2); purified mAbs (Becton Dickinson & Co.) directed against CD16 (Leu-11b) and CD20 (Leu-16); or supernatants containing mAb directed against CD14 (3C10) (16). Immunofluorescent staining was carried out using biotinylated horse anti-mouse IgG (heavy and light chains), and fluorescein-avidin (obtained from Vector Laboratories, Inc.).

Cell were also analyzed by flow cytometry. The buffer used throughout consisted of PBS containing 2% FCS, 0.1% NaN_3, and normal human IgG (1 μM) to block Fc receptor binding. PE-conjugated mAbs reactive with CD3, CD4, CD8, and CD56 (NKH1) were obtained from Coulter Clone (Hialeah, FL). FITC-conjugated mAbs reactive with the IL-2R p55 chain were from Coulter Clone and for the IL-2R p75 chain (18) (TU27) were from Dr. Kazuo Sugamura (Tohoku University, Sendai, Japan). The PE-conjugated and IL-2R p55 mAbs were used at a final dilution of 1:20, and the anti-IL-2R p75 was diluted 1:100. Cells were stained at 4°C for 1 h with two sets of mAbs to reveal nonspecific binding. One set of cells were stained in the presence of a 100-fold excess of unconjugated mAbs, and one set without unconjugated mAbs. After incubation, the cells were centrifuged, the supernatant removed, and 1 ml of PBS + 0.4% paraformaldehyde was added. The cells were stored at 4°C until analyzed by flow cytometry using a cytometer (Ortho Diagnostic Systems, Inc., Westwood, MA). At least 2,000 cells were analyzed in each preparation, and the percentage of positively stained cells was calculated.

Lymphoproliferative Assays. PBMC were prepared by Ficoll-Paque density separation and used to determine responses to the following stimuli: *M. leprae* (10^6 to 10^8 particles/ml) (batch CD-94, obtained from Dr. R. J. W. Rees, Mill Hill, UK, through the WHO Immunology of Leprosy Programme); *M. bovis* Bacillus Calmette-

Guerin sonicate (BCG; 1–10 μg/ml) (19) (Commonwealth Serum Laboratory, Melbourne, Australia); purified protein derivative of tuberculin (PPD; 10 μg/ml) (Staten Serum Institute, Copenhagen, Denmark); PHA (a 1:200 dilution) (Sigma Poole, Dorset, UK). The 65- and 70-kD proteins of BCG (0.1–10 μg/ml) were affinity purified from BCG sonicates using the mAbs L22 and L7, respectively, as described (20, 21), and obtained from the Clinical Immunology Research Center, University of Sydney, Sydney, Australia. The 18-kD recombinant protein of *M. leprae* (0.1–10 μg/ml) (22) was obtained from Dr. Watson, Auckland University School of Medicine, Auckland, New Zealand. The cells were incubated in RPMI 1640 containing penicillin (100 U/ml) (Gibco Laboratories, Grand Island, NY), 2 mM glutamine supplement (Gibco Laboratories), and 10% normal human A^+ serum in a final volume of 220 μl. After 6 d of incubation, the cells were pulsed with 1 μCi [^{3}H]thymidine (Amersham International, Amersham, Bucks, UK). After drying, the filters were prepared for liquid scintillation counting in an liquid scintillation analyzer (1500; Packard Instrument Co., Inc., Downers Grove, IL).

Evaluation of M. leprae Phenolic Glycolipid-1 levels in Patient Serum. Phenolic glycolipid 1 (PGL-1) of *M. leprae* levels in serum of lepromatous patients was evaluated by an ELISA described by Cho et al. (23).

Evaluation of Anti-M. leprae Ig Levels. IgM anti-PGL-1 antibodies were measured by ELISA using the glycoconjugate disaccharide-BSA (dBSA; provided by IMMLEP, World Health Organization) as described (24).

IgG antilipoarabinomannan B (LAM-B) antibodies were measured by ELISA using *M. tuberculosis* LAM-B as antigen (provided by Dr. P. J. Brennan, Colorado State University, Fort Collins, CO) using the conditions described previously (24).

Results

Experimental Design

There were two major considerations in the design of this experiment. The first was to use low, nontoxic doses of rIL-2 and to study the local and systemic influences of the lymphokine in considerable depth. The second was to use the intradermal route to allow us an additional parameter for the evaluation of the intensity of the local cell-mediated immune response. The lymphokine was administered twice daily (9 am and 9 pm) at doses of 10 μg, for a total dose of 160 μg in 8 d. Before, during, and at time intervals up to 6 mo after the completion of the schedule, samples of peripheral blood, skin biopsies, slit smears, and clinical analyses were carried out.

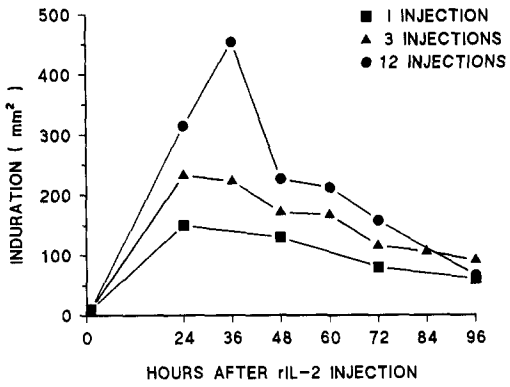


Figure 2. The effect of increasing the dose of rIL-2 on the extent of induration. With an increase in the number of previous IL-2 injections, a larger area of induration is observed at the 24-h time point. However, the kinetics of the decrudescence of the reaction from 48 h were not affected by the dose of IL-2 administered.

The Local Response to rIL-2

The lymphokine was administered in a set pattern on the back starting in the paravertebral area at the level of T_2 and placed ~4 cm on either side of the spinous processes. The 9 am injection was given on the left side and the 9 pm on the right. Injections were then given in a descending fashion

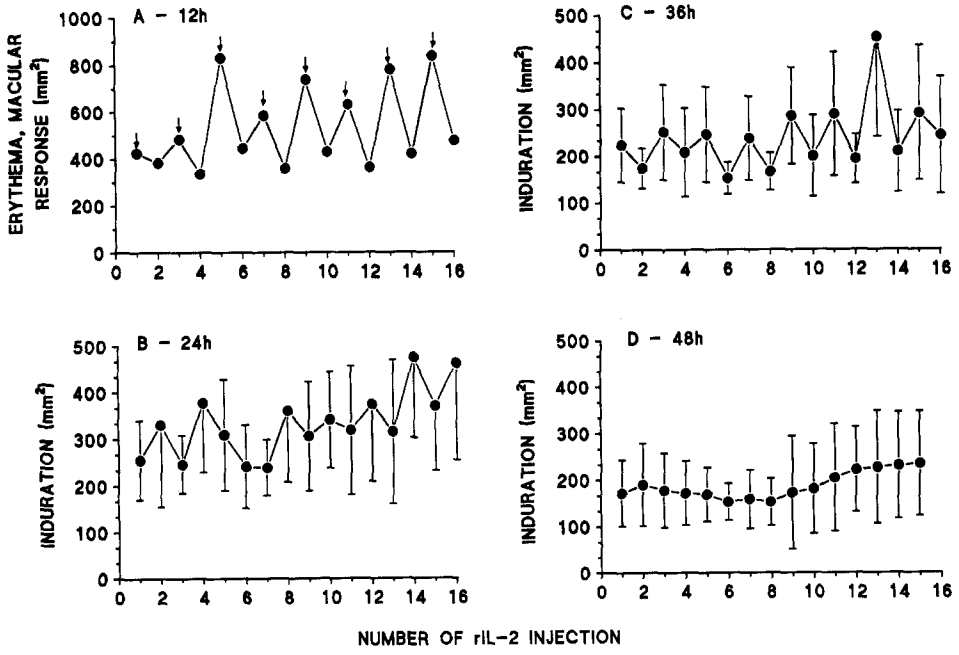


Figure 1. The local response to rIL-2 injections 12 (*A*), 24 (*B*), 36 (*C*), and 48 h (*D*) after lymphokine administration. The mean areas of erythema (*A*) and induration (*B–D*) for all patients tested in mm^2 ± 1 SD of the mean (*B–D*) are shown. At 12 h, a clear diurnal variation was observed with a higher response at sites injected at 9 am. An enhancement in the extent of the response of all sites initially injected at 9 am (*arrows*) was observed with repeated injection. Sites injected at 9 pm gave similar responses throughout the study (*A*). The diurnal variation and the enhancement in the extent of the induration with progressive injections are prominent at 24 h (*B*) and still observed by 36 h (*C*). These changes are no longer evident by 48 h after lymphokine injection (*D*).

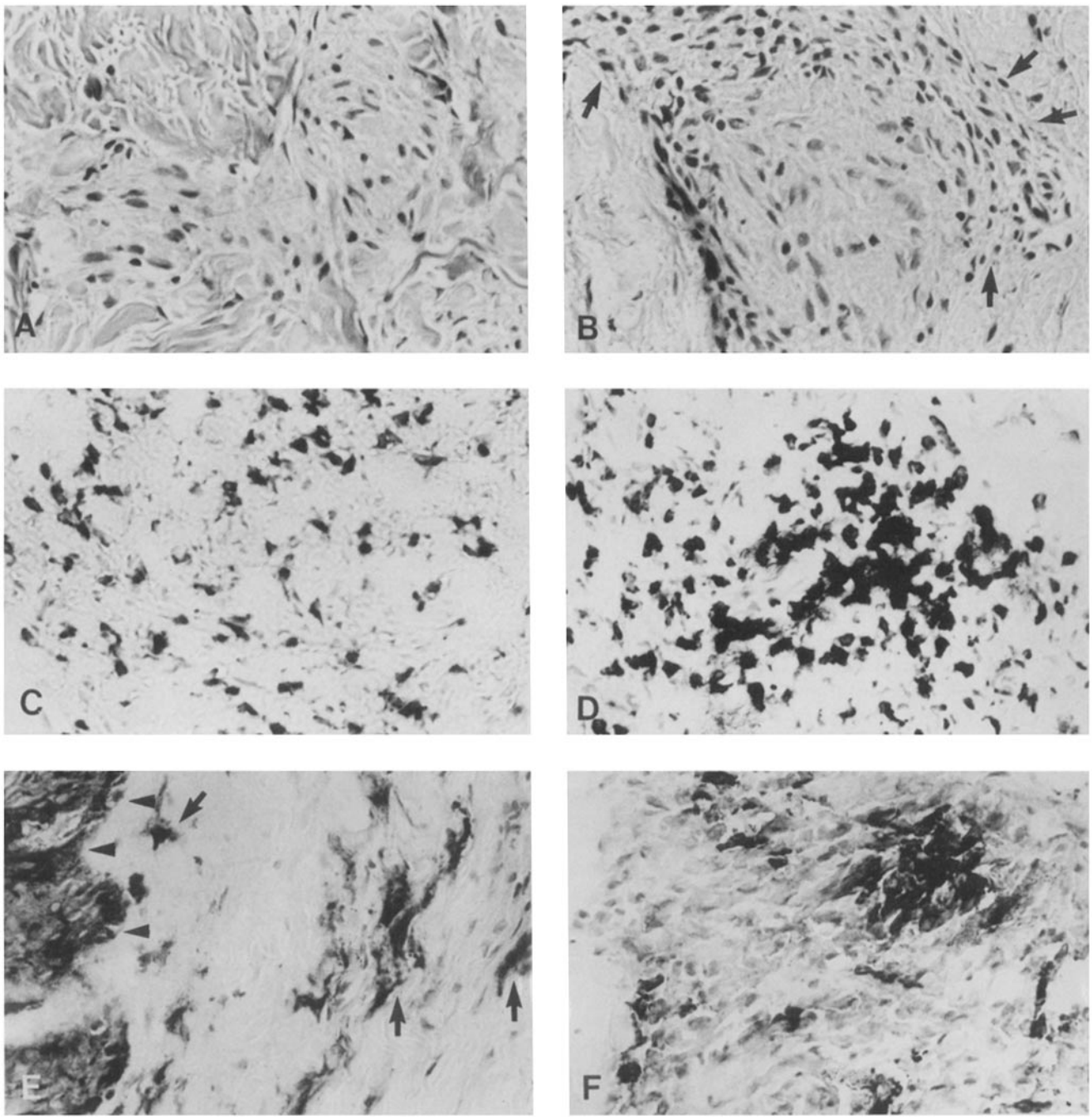


Figure 3. Light microscope micrographs demonstrating the effect of rIL-2 administration on the cellular composition of cutaneous lepromatous leprosy lesions not injected with IL-2 and not from areas within the lymphatic drainage of the injected sites. H+E-stained sections of matched pre-IL-2 (*A*) and post-IL-2 (*B*) skin biopsies revealed an increase in cellularity (mostly lymphoid cells) (*arrows*) after lymphokine injection. Immunohistological staining for $CD3^+$ T cells of matched pre-IL-2 (*C*) and post-IL-2 (*D*) biopsy sites demonstrated a clear IL-2-induced T cell accumulation in the lesions. Immunohistological staining for $CD1^+$ Langerhans cells revealed an IL-2-induced dermal perivascular accumulation of these cells in the post-rIL-2 biopsies (*arrows*) (*E* and *F*). The arrow heads in *E* mark the epidermis. *A* and *B*, patient no. 8, ×50; *C* and *D*, patient no. 11, and *E* and *F*, patient no. 6, ×200.

ending at a site in line with the upper pelvis. The local reactions were never overlapping.

The 12-h Site. The initial injection of 10 μg IL-2 gave an area of induration of ~14 mm in diameter and resembled in every way results obtained in a prior study (5). With repeated injections interesting effects were observed in both the intensity and temporal nature of the local reaction. One of the first to be noted was the striking enhancement in the extent of the response at the 12-h time period (Fig. 1 *A*). This reaction was characterized by the presence of an erythematous, raised, macular lesion with irregular margins and appeared to have a vascular component. At the time of the first

Table 2. *Total and Differential White Cell Counts Before, During and After the Administration of IL-2*

	Days after IL-2 injection*			
	0	5	9	13
Mononuclears	2,349 ± 253	2,669 ± 285	2,952 ± 278	3,552 ± 213
Neutrophils	4,800 ± 425	4,271 ± 429	4,051 ± 434	4,579 ± 632
Eosinophils	634 ± 111	1,314 ± 216	1,041 ± 168	1,472 ± 509
Total white blood cells	7,875	8,278	8,071	9,364

Data represent number of cells per mm^3 of blood.
* 10 μg rIL-2 administered every 12 h intradermally for 8 d for a total dose of 160 μg.
‡ Results are means of bloods obtained from 14 patients ± 1 SEM.

evaluation (12 h), it covered an area of about twice the firm zone of induration found at the 24-h reading (Fig. 1 *B*). With repeated injections, these sites were very much larger with long finger-like extensions extending much beyond the central zone. It became apparent by the end of the series that these larger responses were always the result of the 9 am injection and were enhanced after the fifth injection. This striking diurnal variation in the 12-h response is seen in Fig. 1 *A*.

Indurated Sites. At 24 h and for several days later, the injected sites were firmly indurated. The largest area of induration occurred at 24 h, in keeping with our earlier results. Fig. 1, *B–D* illustrates the sites read at 24, 36, and 48 h. It is clear that with repeated IL-2 injections the maximum zone of induration at 24 h increased by twofold. With the passage of time, both the diurnal variation and local enhancement of induration were less evident. This was the result of the temporal response seen in Fig. 2. Although the maximum zone of induration was markedly affected by increasing the dose of IL-2, the subsequent decrudescence of the reaction occurred with similar kinetics so that by 72 h little difference was noted in the areas of induration.

From our previous studies we know that the 24-h site is already heavily infiltrated with newly emigrated T cells largely of the $CD4^+$ phenotype (5).

Local Skin Lesions. By the end of the course of IL-2 injection, two patients with extensive skin lesions over the entire back showed flattening of the lesions in the central region of the back, whereas more peripheral lesions were unaffected.

The Systemic Effects of rIL-2

Clinical Course. Daily evaluations of the clinical state of patients during and after the 8-d course of IL-2 were performed. Other than mild pruritus, the indurated sites on the back gave no discomfort. A few patients developed a low grade fever (0.6–1.0°C) during the course of the injections, lasting a day or so without malaise. At times after the third day of treatment, nontender axillary lymphodenopathy was present in the majority of patients. Detailed examination of nerve conduction was performed each day by a trained observer. There was no evidence of any additional neural involvement either during or after the study. Neither weight gain nor edema were noted. One patient developed significant fever with swelling and erythema of skin lesions after 13 injections. There was no neural involvement. An acute infection, pharyngitis, had also developed at this time. The rIL-2 was stopped and the cutaneous reversal reaction resolved with corticosteroids.

Peripheral Blood Cells and their Phenotypes. The total and differential cell counts of peripheral blood obtained at times up to 13 d after the initiation of IL-2 injections are shown in Table 2. A small enhancement in total white cells was noted, the majority of which was contributed by a 1.5-fold increase in mononuclear cells and a twofold increase in eosinophils. Neutrophil levels remained unchanged.

The phenotype of PBMC during this same period is shown in Table 3. The number of B lymphocytes and monocytes remained essentially unchanged. T cell numbers increased by about two-fold, with the largest increment being evident in the $CD4^+$ series. It was noteworthy that the number of NK cells increased sixfold starting from subnormal, initial levels. The low numbers of IL-2R bearing cells (the p55 and p75 chains) did not increase.

In Vitro Lymphocyte Function. The responses of PBMC to a variety of antigens expressed on whole *M. leprae* and BCG as well as proteins separated from these organisms is seen in Table 4. Before IL-2 injections, all but one patient failed to respond to *M. leprae* and its 18-kD protein. This nonreactivity persisted in samples collected during and immediately after the entire course of IL-2. One patient demonstrated a positive proliferative response to whole *M. leprae* immediately after the course of rIL-2 injections, but this reverted to negative in the assays at 2 and 6 mo. By comparison, most patients initially responded to whole BCG, PPD, and the 65- and 70-kD proteins of BCG. After IL-2 injections, the cells from most patients showed an enhanced response to these antigens by factors of two- to fourfold. Therefore, it appeared that IL-2 enhanced lymphocyte reactivity to antigens that the patients had been previously sensitized to, but did not modify the selective anergy to *M. leprae*.

The cytotoxic activities of NK and LAK cells before, during, and after IL-2 injections were not appreciably altered.

Table 3. *The Phenotype and Numbers of PBMC Before, During, and After rIL-2 Administration*

Surface antigen (cell type)	Days after IL-2 injection* 0 (14)	5 (5)	9 (4)	13 (5)‡
CD 3 (pan T)	910 ± 129	1,196 ± 266	1,291 ± 124	1,946 ± 615
CD 4 (T helper)	483 ± 155	601 ± 154	863 ± 125	1,252 ± 299
CD 8 (T cytotoxic)	440 ± 116	496 ± 242	929 ± 411	996 ± 88
CD 20 (B cells)	317 ± 103	387 ± 186	256 ± 84	542 ± 96
CD 14 (macrophages)	352 ± 115	335 ± 49	417 ± 84	386 ± 94
CD 56 (NK cells)	55 ± 9	137 ± 34	324 ± 81	460 ± 35
MHC class II	382 ± 129	688 ± 163	507 ± 126	824 ± 220

Data represent number of cells per mm^3 of blood.
* 10 μg rIL-2 administered every 12 h intradermally for 8 d for a total dose of 160 μg.
‡ Results are means of bloods obtained from the numbers of patients in parenthesis ± 1 SEM. The pre-IL-2 values for the three different groups (days 5, 9, and 13) were not significantly different from the mean for all 14 patients.

Table 4. *The Proliferative Response of PBMC to Mycobacterial Antigens Before and After rIL-2 Injection*

	Pre-IL-2 proliferation*						Post-IL-2 percent activity‡					
	M. leprae		BCG				*M. leprae*		BCG			
Patient no.	Whole	18 kD	Whole	65 kD	70 kD	PPD	Whole	18 kD	Whole	65 kD	70 kD	PPD
1	–	–	+	+	+	+	–	–	317	270	152	155
3	–	–	+	+	+	+	299§	–	NT	144	377	NT
6	–	–	+	+	+	+	–	400	381	514	314	211
8	–	–	+	+	+	+	–	–	383	374	253	234
9	–	–	+	+	+	+	–	–	242	219	34	204
10	–	–	±	±	–	–	–	–	155	189	–	–
11	–	–	+	+	+	+	–	–	163	731	280	186
13	–	–	+	+	+	+	–	–	155	413	235	187
2	–	–	+	+	+	+	–	–	76	77	61	66
5	–	–	+	+	+	+	–	–	33	31	48	157
14	+	–	+	+	+	+	93	–	50	78	94	129
4	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–

* [^{3}H]TdR incorporation was considered + when the stimulation index was >10, ± when the stimulation index was 6–10, and – when <5.
‡ Activity was calculated as percent of [^{3}H]TdR incorporation 5–13 d after initiation of IL-2 injection (see Table 1) evaluated as stimulation index relative to the pre-IL-2 response.
§ A transient enhancement in proliferative response to whole *M. leprae* was observed 1 d after the last IL-2 injection (9 d). Responsiveness was negative when the patient was tested 2 mo later.

Table 5. *Effect of IL-2 Administration on the Histology, Cellular Phenotype and, M. leprae Load in the Skin*

	Histological diagnosis			Enhanced T cell numbers*	Slit smears (six sites)			Histological biopsies			
Patient no.	Pre-IL-2	2 mo	6 mo	5–13 d	Pre-IL-2	2 mo	6 mo	Pre-IL-2	5–13 d	6 mo	6 mo DB‡
					B. I.				*B. I.*		
1	LL	BL§	LL	+/−‖	4.0	4.1	3.3	4+	4+	4+	3+
2	LL	LL	LL	+	3.8	3.8	3.9	5+	5+	4+	4+
3	LL	LL	LL	−	4.3	3.6	4.3	5+	5+	6+	5+
4	LL	LL	BL	+/−‖	4.3	3.5	3.7	5+	5+	4+	1+
5	LL	LL	LL	−	4.3	3.5	4.0	5+	5+	5+	4+
6	LL	LL	SLL_s	+	3.8	3.2	4.0	5+	5+	4+	4+
7	LL	BL§	ND	+	5.0	4.5	ND	6+	5+	4+	4+
8	LL	BL§	BL	+	3.7	3.5	3.0	5+	4+	4+	4+
9	LL	LL	LL	±	4.7	4.3	3.8	5+	6+	5+	1+
10	BL	BT§	ND	+/−‖	2.6	2.2	ND	2+	0	ND	ND
11	LL	LL	LL	+	4.7	ND	4.2	6+	5+	6+	5+
12	BL	BT§	negative	+	3.3	2.8	1.4	3+	1+	0+	0+
13	LL	LL	BL	+/−‖	3.5	ND	2.6	6+	5+	5+	ND
14	LL	LL	ND	+	3.6	3.1	ND	2+	2+	ND	ND

* T cell numbers were evaluated by direct microscopic counting of H + E-stained and immunohistological stained sections. (+). The relative numbers of T cells were at least doubled by both methods of evaluation; (±) slight enhancement in T cell numbers was observed; (−) no change in T cell numbers was observed.

‡ 6-mo post-IL-2 injection biopsy taken from a site on the back distal to the IL-2 injection area.

§ Biopsies that showed upgrading and the presence of epithelioid and multinucleated giant cells.

‖ T cell numbers enhanced by H + E staining but not by immunohistological evaluation.

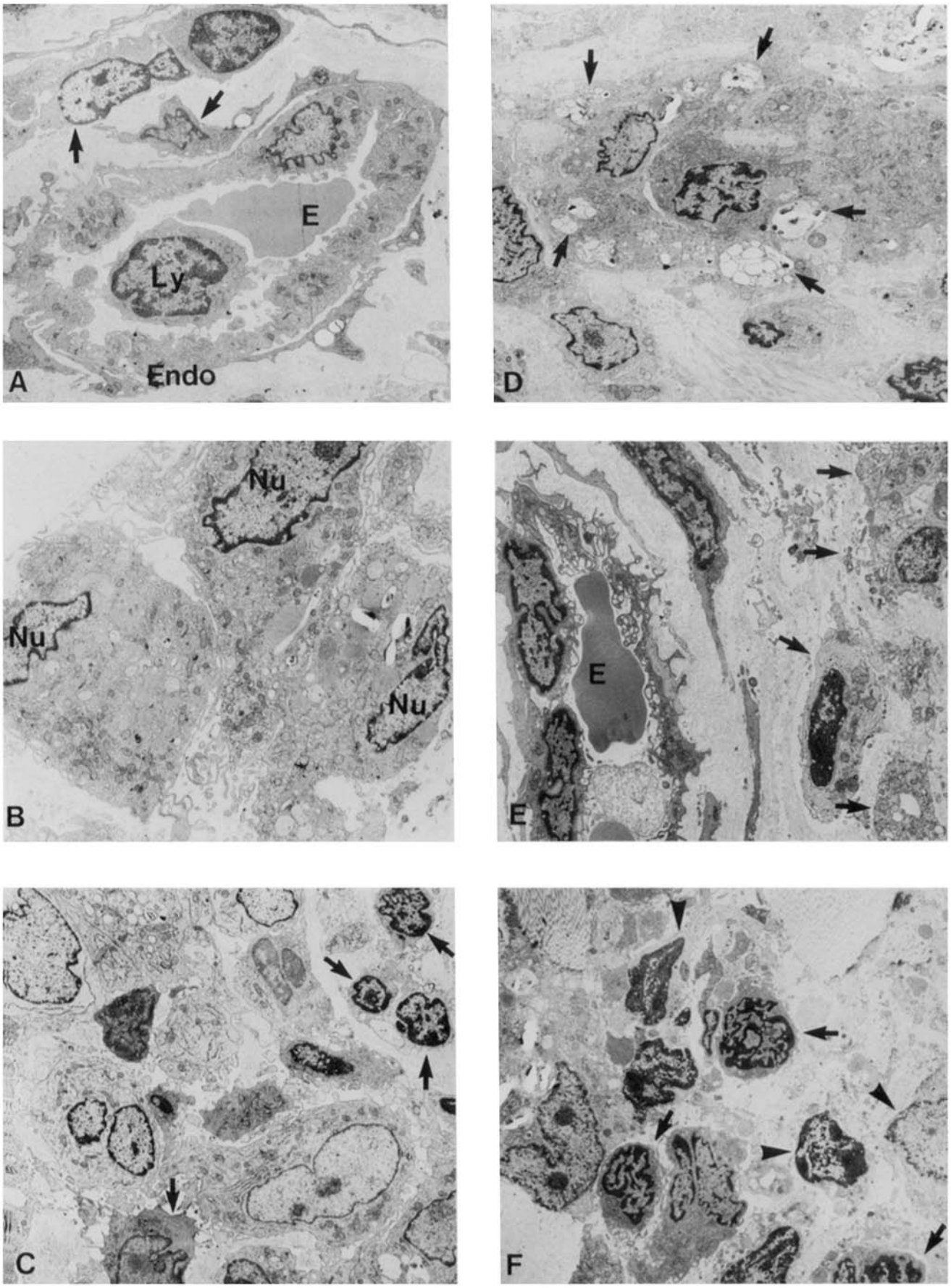
Immunocytochemistry and Histopathology of Peripheral Sites. We have previously described the local response to IL-2 inoculation, including the numbers and phenotypes of emigratory cell populations (5). In this study, we focused on changes at matching skin sites distal from the foci of IL-2 injections (sites not injected with nor in the area of drainage of IL-2).

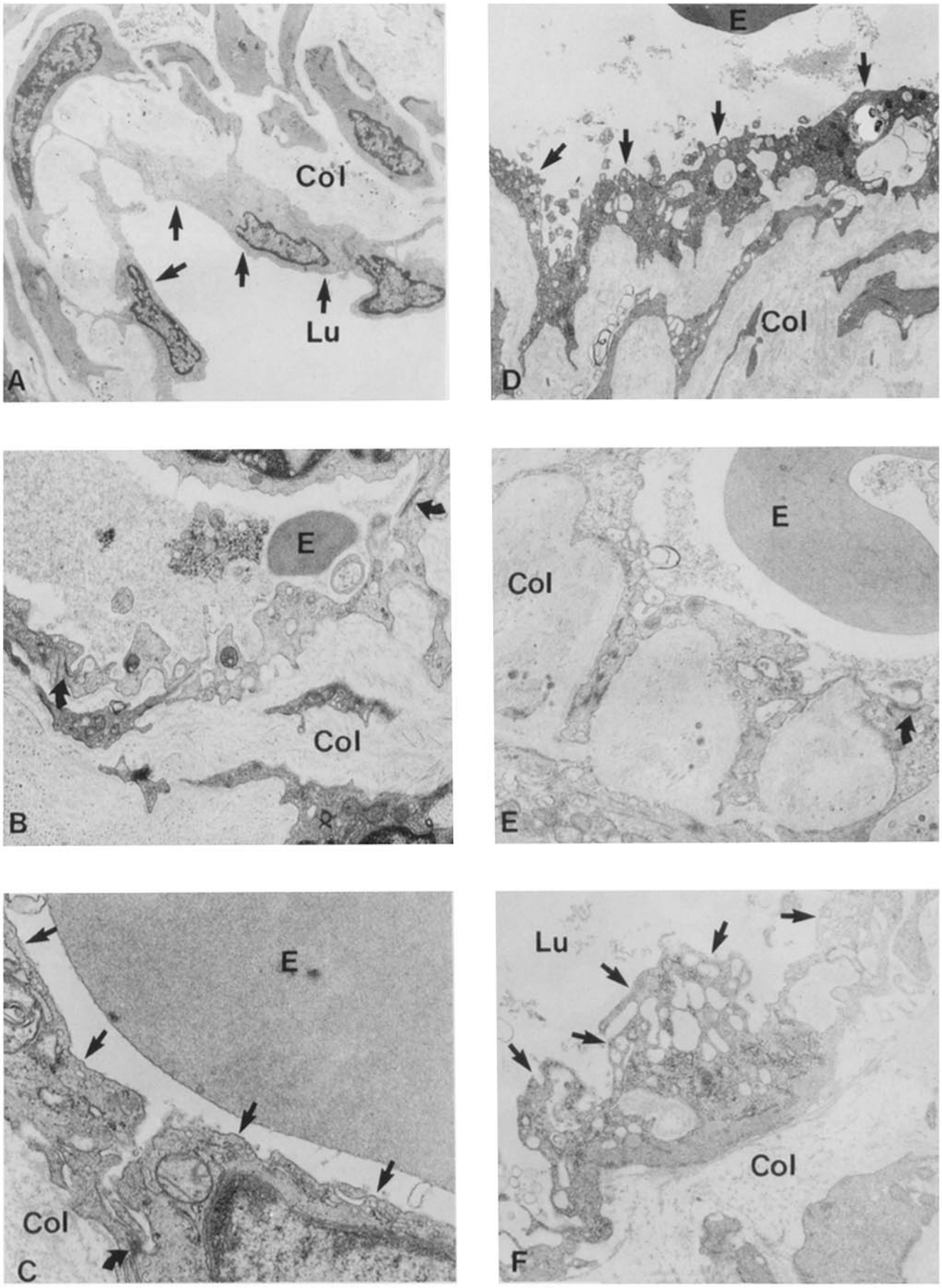
Within 13 d after the administration of rIL-2, skin biopsies of 12 of 14 patients demonstrated an increase in lymphoid cells at peripheral sites (Table 5 and Fig. 3, *A* and *B*). By mAb analysis, the emigratory cells consisted largely of T lymphocytes, and $CD4^+$ T cells were present in large numbers (Fig. 3, *C* and *D*). This body-wide infiltrate also contained newly arrived monocytes and macrophages with the presence of differentiated epithelioid cells and multinucleated giant cells. Of interest was the increase in the number of CD1-positive Langerhans cells in the dermal infiltrate (Fig. 3, *E* and *F*) in a perivascular localization. Endothelial cells of the skin vasculature expressed MHC class II antigens on their surface (data not shown).

Transmission Electron Microscopy of Peripheral Sites. Glutaraldehyde-fixed specimens of skin biopsies of distal lesions were taken before and after IL-2 administration. It is clear from Fig. 4 that the cellularity of the post-IL-2 sites was considerably increased with a preponderance of lymphoid cells. This is in keeping with the H + E-stained sections illustrated in Fig. 3. Parasitized macrophages and foam cells were clearly damaged and fragmented.

An unusual finding was made upon closer examination of the endothelial cells of small dermal vessels. These are seen in more detail in Fig. 5. The endothelium of pre-IL-2 sites

Figure 4. Transmission electron micrographs demonstrating the effect of rIL-2 administration on the cellular response in lesions of lepromatous leprosy patients (see above). (*A*) Pre-IL-2 site containing a small blood vessel with an erythrocyte (*E*) and a lymphocyte (*Ly*). A loose perivascular infiltrate of inflammatory cells is observed (*arrows*). The endothelium (*Endo*) appears normal. (*B* Pre-IL-2 site from the same biopsy as *A*. The inflammatory infiltrate is composed predominantly of parasitized foamy macrophages. Three such cells with their nuclei (*Nu*) can be seen. (*C*) Post-IL-2 site from a matched lesion obtained from the same patient as in *A* and *B*. The cellularity of the lesion is much higher. More lymphoid cells are present (*arrows*) and some of the macrophages have a more epithelioid appearance. (*D*) Pre-IL-2 site containing macrophages with intravacuolar *M. leprae* (*arrows*). (*E*) Post-IL-2 injection site showing a small dermal blood vessel identified by erythrocytes (*E*) with extensive vacuolization of the endothelium. The perivascular phagocytes appear damaged and disintegrated (*arrows*). (*F*) Post-IL-2 injection site showing damaged macrophages (*arrow heads*) and intact lymphoid cells (*arrows*). *A–C*, patient no. 12, *D–F*, patient no. 8. *A* and *B*, ×5,000; *C* and *D*, ×3,000; *E*, ×4,000; *F*, ×3,200.


A
E
Ly
Endo
D
B
Nu
Nu
Nu
E
E
C
F


Col
Lu
A
E
Col
D
E
Col
B
E
Col
E
Lu
Col
F
E
Col
C

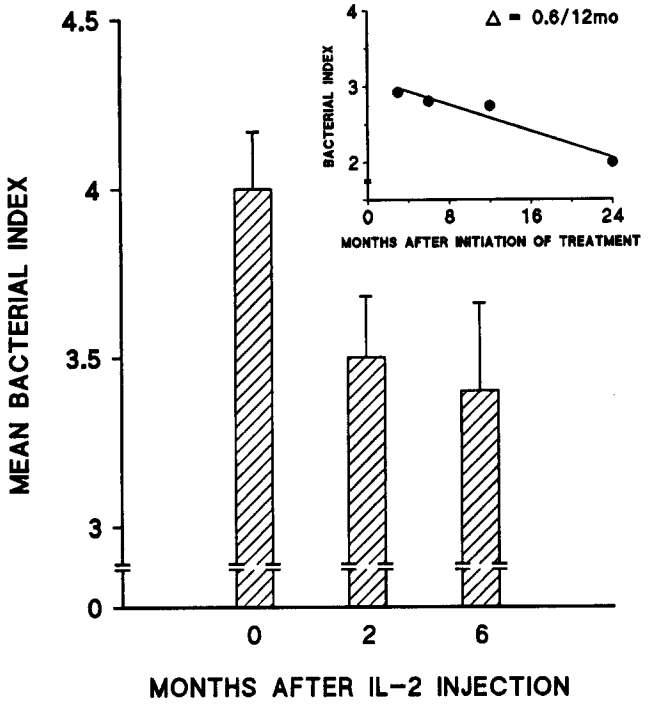


Figure 6. The reduction in bacterial index evaluated from slit smears of the same six sites after rIL-2 injection. The B.I. of patients was evaluated before (0), at 2, and at 6 mo after IL-2 injection. The mean ± 1 SEM is shown for all patients tested at each time point. The insert shows the B.I. reduction for 30 matched historical controls. A 0.6 log reduction is observed during 12 mo of therapy (see Materials and Methods).

is rather flat and contains numerous small endocytic vessels in keeping with the usual structure of capillaries (Fig. 5, *A–C*). After IL-2 injection, the endothelium became thickened with many long projections extending into the lumen, and larger cytoplasmic vacuoles were evident (Fig. 5, *D–F*). Emigratory cells were observed between endothelial cells, and the lumen contained larger numbers of mononuclear leukocytes. The basement membrane of the vessels was thickened and had an edematous appearance. Extravascular sites were essentially normal other than their increased cellularity, and a striking selective destruction of foam cells and other mononuclear phagocytes.

The Fate of M. Leprae. For slit smear analysis, the same, six sites were tested at various time points before and after IL-2 injection, and the B.I. of the sites were reviewed by trained technical staff. The composite data on 14 patients is given in Table 5. Upon slit smear analysis, 7 of 12 patients evaluated demonstrated 0.5 log or greater reduction in B.I. at 2 mo. A mean reduction of 0.5 log units for all patients tested at 2 mo was observed (Table 5 and Fig. 6). An additional 0.1 log units reduction in B.I. was noted from 2 to 6 mo, suggesting that the effect of IL-2 injection on bacterial clearance was almost immediate and required the continuous presence of the lymphokine. Matched historical control BL/LL leprosy patients, not injected with IL-2, were evaluated for B.I. reduction during 24 mo of MDT. These controls demonstrated a 0.5–0.6 log unit reduction in B.I. during 12 mo of MDT or 0.08–0.1 log units per 2 mo of therapy (Fig. 6). Thus, consistent with these results from matched historical controls, a five- to sixfold increase of clearance was observed during the 2 mo after only 8 d of IL-2 injection.

For biopsy analysis, examination of peripheral skin biopsies was performed before IL-2 injections, 5–13 d after the initiation of IL-2, and at 2 and 6 mo thereafter. These were obtained from leprosy lesions not injected with IL-2 and not within the lymphatic drainage of the injected sites (primarily the arms and legs). Coded slides were examined for histological diagnosis, lymphocytic infiltrate, and bacillary indices by two independent investigators.

Bacterial indices evaluated from biopsy sections showed a 1 log or greater reduction in 6 of 14 patients already at the 5–13-d time point (Table 5 and Fig. 7). 6 mo after the course of IL-2 injection, 7 of 12 patients showed B.I. reductions, at peripheral sites not exposed directly to IL-2 (Table 5, Fig. 8). Biopsy sites on the back showed a more striking decrease in bacillary load than elsewhere on the body. This zonal effect may be related to the lymphatic drainage of the area and the passage of a greater number of inflammatory cells from the centrally located IL-2 sites.

Antibody Titers against M. leprae Components. Serum levels of PGL-1 were measured before and at various times after IL-2 injection. Antigen levels were high in the tested patients at the start of the study. However, after the initiation of IL-2 injection, a sharp reduction in the levels of antigen in the serum was observed even within 2 wk of treatment. (Fig. 9 *A*).

Antibody titers against *M. leprae* PGL-1 and the polysaccharide antigen LAM-B were measured before and at various times after IL-2 injections. All but one of our Nepalese patients had been treated with a multidrug regimen for 1–6 mo, and the IgM PGL-1 levels had fallen from pretreatment levels in some cases. 2 mo after IL-2 injections, there was a rise in the IgM anti-PGL-1 levels, and by 6 mo, the levels were falling (Fig. 9 *B*). The IgG anti-LAM-B levels increased about twofold only (data not shown).

We suggest that the lymphokine-enhanced degradation and clearance of bacilli reduces the amount of *M. leprae* antigens in the circulation. It also stimulates the synthesis of antibodies directed at mycobacterial cell wall carbohydrates and lipids.

Figure 5. Transmission electron micrograph demonstrating the effect of rIL-2 administration on the small blood vessels of lepromatous lesions (see Fig. 4). The endothelial surface forming the lumen (*Lu*) of the pre-IL-2 sites (*A–C*) is relatively flat and regular (*arrows* in *A* and *C*) and contains many small, regular endocytic vesicles. The underlying collagen (*coll*) is normal. In the post-IL-2 lesions (*D–F*), the endothelium is swollen with long projections into the vessel lumen (*arrows*) and large irregular vacuoles. The underlying basement membrane is thickened and edematous (*coll*) E-erythrocytes. The junctions of the vasculature appear intact both before (*B* and *C*) and after (*E*) IL-2 injection (*curved arrow* in *B*, *C*, and *E*). *A*, *D*, and *F*, patient no. 11; *B*, *C*, and *E*, patient no. 14. *A*, ×4,600; *B*, ×9,000; *C*, ×20,000; *D*, ×6,600; *E*, ×3,700; *F*, ×11,000.

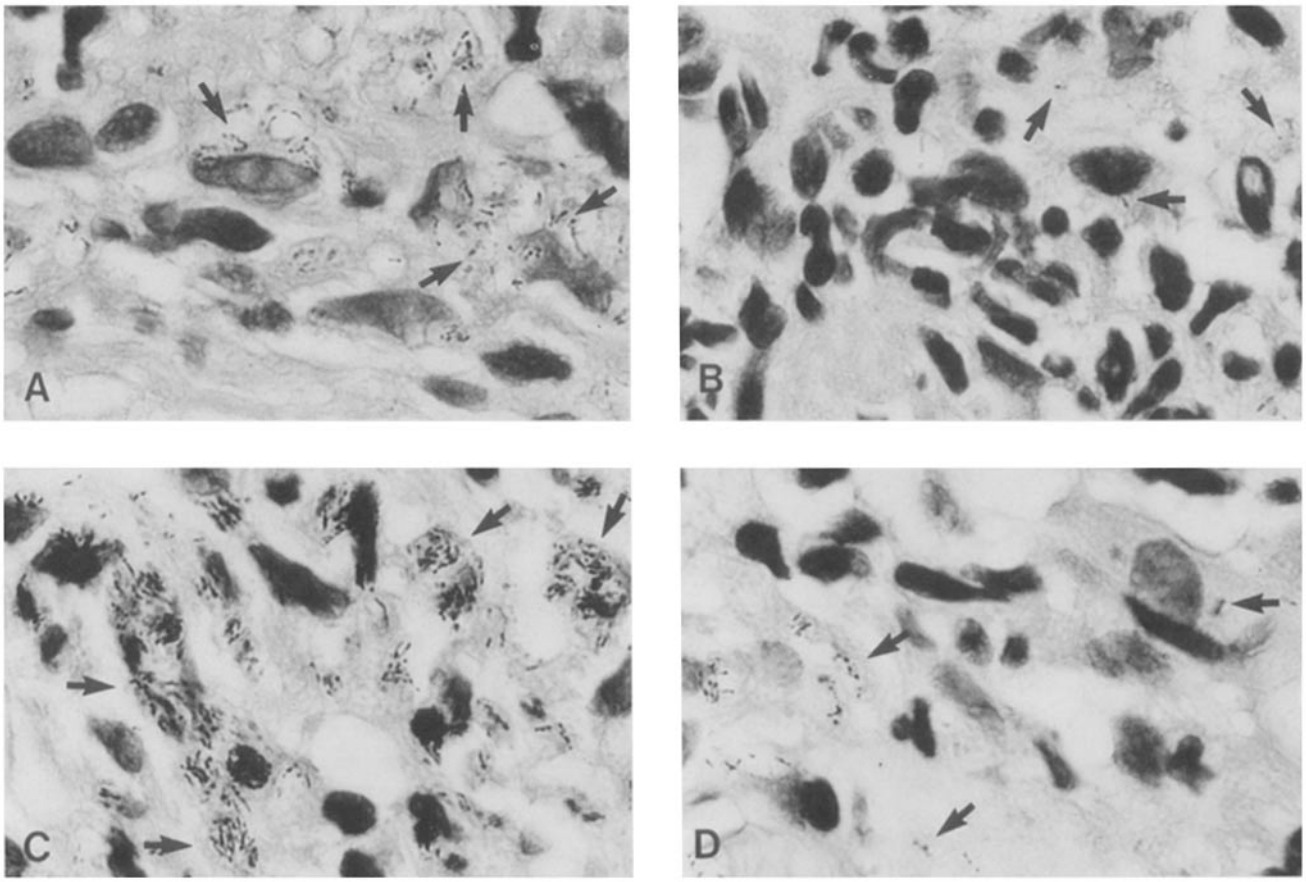


Figure 7. Effect of IL-2 administration on the *M. leprae* load in the lesions of lepromatous patients taken from sites not injected with IL-2 and not within areas of lymphatic drainage of the injected sites. High numbers of acid fast bacilli are seen is the pre-IL-2 sites (*arrows*) (*A* and *C*). A clear reduction in the numbers of bacilli of matched site after IL-2 injection is observed (*arrows*) (*B* and *D*, respectively). *A* and *B*, patient no. 7; *C* and *D*, patient no. 8. (×400) (fite acid-fast stain).

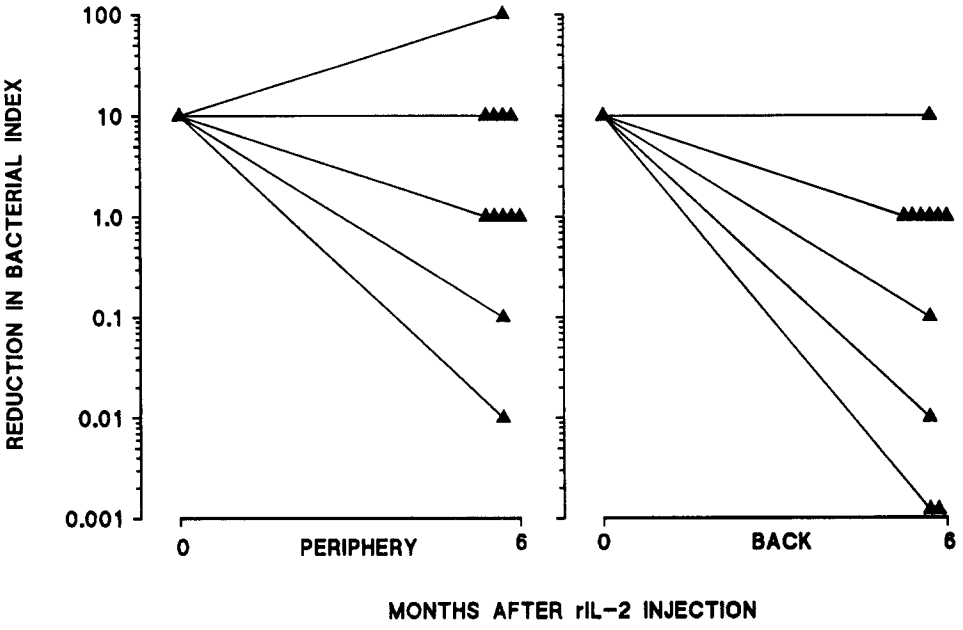


Figure 8. The reduction in B.I. evaluated from acid-fast stained sections of biopsies after rIL-2 injection. The B.I. of patients was evaluated before (0) and 6 mo after the initiation of IL-2 injections. Biopsies were taken from lesions in the periphery not injected with IL-2 and not in areas of IL-2 drainage or sites on the back not directly injected with IL-2 but possibly in areas of IL-2 drainage. Each line represents a single patient.

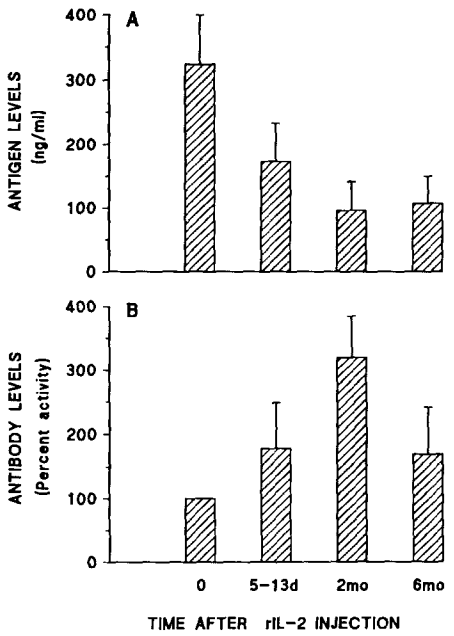


Figure 9. The effect of IL-2 injection on the levels of PGL-1 antigen (*A*) and on IgM levels against PGL-1 (*B*). Activity was evaluated in the serum of patients before (0), 5–13 d, 2 mo, and 6 mo after start of IL-2 injection.

Discussion

This limited trial of rIL-2 administered intradermally has successfully modified the natural history of leprosy in the majority of patients. Low doses of the lymphokine have had clear systemic effects, including an elevation of the number of circulating mononuclear cells, a generalized infiltration of lymphocytes into the skin, and the subsequent rapid clearance of *M. leprae* (fivefold increase in the rate of clearance) as compared with the influence of MDT alone. This occurred in the absence of significant IL-2 toxicity, and without precipitating an uncontrolled reactive state or additional peripheral neuropathy. Moreover, it has been achieved in an accelerated time frame measured in weeks rather than in years.

Our findings indicate that rIL-2 has enhanced the generation of a more vigorous cell-mediated immune response in patients with lepromatous leprosy. This response has occurred without indication that we have stimulated specific anti–*M. leprae* cellular immunity, in that neither *M. leprae*–specific T cell mitogenesis nor the generation of cytotoxic effector cells to *M. leprae* antigen occurred. Even so, enhanced cellular reactivity was characterized by the: progressively increased zones of induration at the injection sites; generalized cutaneous infiltration with T cells, monocytes, and Langerhans cells; a reduction in the bacillary index as determined by slit smears and biopsies; and a reduction in the size of skin lesions. The elevation in antibodies against *M. leprae* products is consistent with this interpretation and suggests a humoral immune response to a new antigenic load released from the tissues. In addition, the reduction in *M. leprae* PGL-1 antigen levels is consistent either with a systemic reduction in bacillary load and/or the clearance of immune complexes.

The progressive increase in the zones of induration taking place during the course of IL-2 injections is the result of an increased number of emigratory cells entering the sites. This is in keeping with previous information on the relationship between induration, the percentage of the dermis infiltrated, and the percentage of T cells in IL-2 and antigen-driven reactions (2, 5). In a manner yet to be determined, prior IL-2 injections influence the intensity of the local emigratory response. This may occur by sensitizing circulatory cells for the recognition of the local site and/or by modifying the endothelium of the local vasculature. As we observed, the endothelium of small, dermal vessels is structurally altered and expresses MHC determinants. This may promote the binding and transmigration of intraluminal mononuclear cells and may help explain the widespread influx of T cells into peripheral sites. One must also consider roles for other IL-2-induced lymphokines and cytokines (e.g., IFN-γ) in generating local chemoattractants. We suspect that the efficacy of the emigratory response is in part the result of the pre-existing infiltrates that occur in the skin of lepromatous leprosy patients. Preliminary observations in patients with a normal dermal milieu, but receiving long-term IL-2 therapy, show smaller numbers of infiltrating cells (G. Ritz, personal communication). Finally, the 12-h responses are indicative of enhanced vascular permeability and functional alterations in the endothelium.

The reduction in the number of leprosy bacilli that occurs in both local and peripheral sites requires the destruction of aged, parasitized macrophages (3, 5). This process is associated with the generation of effector cells that selectively recognize infected mononuclear phagocytes and spare other elements of the cutaneous site. Since we cannot document the enhancement of *M. leprae*–specific cytotoxic T cells, it is likely that cells with less selective recognition mechanisms may be involved. These would include NK, LAK, and perhaps γ/δ^+ T cells as likely candidates for this mechanism (7, 25). These findings are consistent with prior results from the analysis of the cellular composition of antigen-initiated DTH responses: by far, the vast majority of cells emigrating to the site of antigen inoculation are not antigen-specific reactive T cells. From the results of this study, it appears that IL-2 or an IL-2-induced product can act as a chemotactic signal for extravasation of mononuclear leukocytes into the dermis, where these probably mediate the antimicrobial response we have observed.

These results support the conclusion that IL-2 can reduce the total body burden of *M. leprae* when combined with multiple drug chemotherapy in patients with leprosy. This effect appears to result from a controlled enhanced cell-mediated response and may be applicable to a number of other chronic infectious processes, including the opportunistic pathogens of AIDS. The partial responses obtained after only 8 d suggest that more prolonged administration of the lymphokine would be beneficial and might even be curative. Whether or not this form of immunotherapy will be applicable to a larger segment of leprosy patients is unclear. However, it is evident that a detailed analysis of lymphokine biology in man is central to our ability to manipulate the immune response in the future.

We thank the patients and staff of Anandaban Leprosy Hospital, especially Dr. Alice Theuvenet, Sister Nobuko Miyazaki, Miss Kathleen Finlay, and Mrs. Sarah Failbus. We thank A. R. de Moura for the graphic work and M. Garcia for efficient secretarial help.

This study was supported by U.S. Public Health Service grant AI-22616 and by Cetus Corporation. Anandaban Leprosy Hospital is fully supported by The Leprosy Mission International. G. E. Hancock was a Heiser Program for Research in Leprosy fellow. LAM-B was prepared by Dr. Brennan under National Institutes of Health contract AI-52582.

Address correspondence to Gilla Kaplan, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Received for publication 20 December 1990.

References

1. Kaplan, G., M.D. Widmer, I. Nath, R.M. Steinman, S. Laal, H.K. Prasad, E.N. Sarno, U. Elvers, and Z.A. Cohn. 1986. Influence of delayed immune reactions on human epidermal keratinocytes. *Proc. Natl. Acad. Sci. USA.* 83:3469.
2. Kaplan, G., S. Laal, G. Sheftel, A. Nusrat, I. Nath, N.K. Mathur, R.S. Mishra, and Z.A. Cohn. 1988. The nature and kinetics of a delayed immune response to purified protein derivative of tuberculin in the skin of lepromatous leprosy patients. *J. Exp. Med.* 168:1811.
3. Kaplan, G., G. Sheftel, C. K. Job, N.K. Mathur, I. Nath, and Z.A. Cohn. 1988. Efficacy of a cell-mediated reaction to the purified protein derivative of tuberculin in the disposal of mycobacterium leprae from human skin. *Proc. Natl. Acad. Sci. USA.* 85:5210.
4. Kaplan, G., A.D. Luster, G. Hancock, and Z.A. Cohn. 1987. The expression of a γ interferon-induced protein (IP-10) in delayed immune responses in human skin. *J. Exp. Med.* 166:1098.
5. Kaplan, G., R. Kiessling, S. Teklemariam, G. Hancock, G. Sheftel, C.K. Job, P. Converse, T.H.M. Ottenhoff, M. Becx-Bleumink, M. Dietz, and Z.A. Cohn. The reconstitution of cell-mediated immunity in the cutaneous lesions of lepromatous leprosy by recombinant interleukin 2. *J. Exp. Med.* 169:893.
6. Converse, P., T.H.M. Ottenhoff, S.W. Teklemariam, G.E. Hancock, M. Dietz, M. Becx-Bleumink, A. Wondimu, R. Kiessling, Z.A. Cohn, and G. Kaplan. 1990. Intradermal recombinant interleukin 2 enhances peripheral blood T-cell responses to mitogen and antigens in patients with lepromatous leprosy. *Scand. J. Immunol.* 32:83.
7. Hancock, G.E., A. Molloy, B.K. Ab, R. Kiessling, M. Becx-Bleumink, Z.A. Cohn, and G. Kaplan. 1991. In vivo administration of low-dose human interleukin-2 induces lymphokine-activated killer cells for enhanced cytolysis in vitro. *Cell. Immunol.* 132:277.
8. Ridley, D.S., and W.H. Jopling. 1966. Classification of leprosy according to immunity: a five group system. *Int. J. Lepr.* 34:255.
9. Ridley, D.S., and G.R.F. Hilson. 1967. A logarithmic index of bacilli in biopsies. 1. Method. *Int. J. Lepr.* 35:184.
10. Ridley, D.S. 1967. A logarithmic index of bacilli in biopsies. 2. Evaluation. *Int. J. Lepr.* 35:187.
11. Mclean, I.W., and P.K. Nakane. 1974. Periodate-lysine-paraformaldehyde fixative: a new fixative for immunoelectron microscopy. *J. Histochem. Cytochem.* 22:1977.
12. Engelman, E.G., R. Warnke, R.I. Fox, and R. Levy. 1981. Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* 78:1791.
13. Evans, R.R., D.W. Wall, C.D. Platsoucas, E.P. Siegal, S.M. Fikrig, C.M. Testa, and R.A. Good. 1981. Thymus-dependent membrane antigens in man: inhibition of cell mediated lympholysis by monoclonal antibodies to the TH_2 antigen. *Proc. Natl. Acad. Sci. USA.* 78:544.
14. Schwarting, R., H. Stein, and C.Y. Wang. 1985. The monoclonal antibodies anti S-HC1-1 and anti-S-HC1-3 allow the diagnosis of hairy cell leukemia. *Blood.* 65:974.
15. Fithian, E., P. King, G. Goldstein, M. Rubenfeld, C. Fenoglio, and E. Edelson. 1981. Reactivity of Langerhans' cells with hybridoma antibody. *Proc. Natl. Acad. Sci. USA.* 78:2541.
16. Van Voorhis, W.C., R. Steinman, L. Hair, J. Luban, M. Witmer, and Z.A. Cohn. 1983. Specific mononuclear phagocyte monoclonal antibodies. Application to the purification of dendritic cells and tissue localization of macrophages. *J. Exp. Med.* 158:126.
17. Luster, A.D., and J.V. Ravetch. 1987. Biochemical characterization of a γ interferon-inducible cytokine (IP-10). *J. Exp. Med.* 166:1084.
18. Teshigawara, K., H.-M. Wang, K. Kato, and K.A. Smith. 1987. Interleukin 2 high-affinity receptor expression requires two distinct binding proteins. *J. Exp. Med.* 165:223.
19. Britton, W.J., L. Hellqvist, A. Basten, and R.L. Raison. 1985. Mycobacterium leprae antigens involved in human immune responses. I. Identification of protein antigens by monoclonal antibodies. *J. Immunol.* 135:4171.
20. Britton, W.J., L. Hellqvist, A. Basten, and A.S. Inglis. 1986. Immunoreactivity of a 70kD protein purified from *mycobacterium bovis* (BCG) by monoclonal antibody affinity chromatography. *J. Exp. Med.* 164:695.
21. Britton, W.J., L. Hellqvist, R.J. Garsia, and A. Basten. 1987. Dominant cell wall proteins of *Mycobacterium leprae* recognized by monoclonal antibodies. *Clin. Exp. Immunol.* 67:31.
22. Booth, R.J., D.P. Harris, J.M. Love, and J.D. Watson. 1988. Antigenic proteins of mycobacterium leprae: complete sequence of the gene for the 18kDa protein. *J. Immunol.* 140:597.
23. Cho, S.N., S.W. Hunter, R.H. Gelber, T.H. Rea, and P.J. Brennan. 1986. Quantitation of the phenolic glycolipid of *Mycobacterium leprae* and relevance to glycolipid antigenemia in leprosy. *J. Infect. Dis.* 153:560.
24. Roche, P.W., W.J. Britton, S.S. Failbus, H. Ludwig, W.J. Theuvenet, and R.B. Adiga. 1990. Heterogeneity of serological responses in paucibacillary leprosy: differential responses to protein and carbohydrate antigens and correlation with clinical parameters. *Int. J. Lepr.* 58:319.
25. Nakata, M., M.J. Snyth, Y. Norihisa, A. Kawasaki, Y. Shinkai, K. Okumura, and H. Yagita. 1990. Constitutive expression of pore-forming protein in peripheral blood γ/δ T cells: implication for their cytotoxic role in vivo. *J. Exp. Med.* 172:1877.