

THE RECONSTITUTION OF CELL-MEDIATED IMMUNITY  
IN THE CUTANEOUS LESIONS OF LEPROMATOUS  
LEPROSY BY RECOMBINANT INTERLEUKIN 2

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The basic defect in lepromatous leprosy is the selective unresponsiveness of T cells to the antigens of *Mycobacterium leprae* (1). This defect may be partial or complete (2) and does not appear to change with prolonged chemotherapy (3). A lack of T cell-derived lymphokines such as IFN- $\gamma$  and IL-2 could account for the inability of macrophages and other cells to eliminate *M. leprae*.

Attempts to circumvent T cell unresponsiveness have already been initiated in both in vitro systems (2, 4-6) and in the confines of the cutaneous lesions. Initial observations have been reported on the efficacy of crossreacting antigens such as purified protein derivative of tuberculin (PPD)<sup>1</sup> and the lymphokine rIFN- $\gamma$  (7-10). In the case of PPD, intradermal administration has led to a long-lived emigratory mononuclear leukocyte response, the local destruction of parasitized macrophages, and a striking reduction in the number of *M. leprae* within 21 d. These responses and the accompanying modification of cellular subsets are evidence of a vigorous cell-mediated immune reaction in the environment of a lepromatous lesion. Similar observations have been made after IFN- $\gamma$  administration (manuscript in preparation).

We have now extended these observations to the use of human rIL-2. This lymphokine serves as an autocrine T cell growth factor, and induces the formation of lymphokines with other functions (11, 12). We report results on the use of small doses of IL-2, in the skin of lepromatous patients, and the reconstitution of cutaneous cellular immunity.

### Materials and Methods

*Patient Population.* 35 patients, 18-60 yr old, 23 with lepromatous leprosy (LL) and 12 with borderline lepromatous leprosy (BL) (13), were selected for the intradermal administra-

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<sup>1</sup> Abbreviations used in this paper: BI, bacterial index; BL, borderline lepromatous leprosy; DTH, delayed-type hypersensitivity; LL, lepromatous leprosy; PPD, purified protein derivative of tuberculin.

tion of rIL-2 (Cetus Corp., Emeryville, CA). Patients were referred to the study from the out-patient clinic at the All Africa Leprosy Rehabilitation and Training Centre (ALERT, Addis Ababa). Some of the patients were hospitalized or newly diagnosed patients. 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 wk to 5 yr, and had received multidrug therapy with Rifampin (600 mg/d), Dapsone (100 mg/d) and about half received Clofazimine (50–100 mg/d and/or 300 mg/mo) in addition.

Patients were admitted to the ALERT Addis Ababa Leprosy Hospital and routine laboratory tests were performed on blood, urine, and stools. A general clinical and neurological exam was conducted. All chemotherapy was continued during the trial. Slit smears for bacterial index (BI) evaluation were performed a day or two before IL-2 injection. The BI of patients tested ranged from 3 to 5+.

**IL-2 Administration.** Human rIL-2 ( $3 \times 10^6$  U/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 or 250 µg/ml and used within 2 h of reconstitution. Eight patients received a single dose of 10 µg rIL-2, six received a single dose of 25 µg rIL-2, four received two 10-µg doses at 48-h intervals, and 17 received three 10-µg doses at 48-h intervals. Patients receiving one to two doses of IL-2 were injected in apparently normal skin on the lower back, while patients injected with three doses were tested in macular or nodular lepromatous lesions. Local erythema and induration of the injected site were evaluated daily. These studies were carried out at the Armauer Hansen Research Institute (AHRI), Addis Ababa. The site of IL-2 injection and a matched control site were biopsied (4-mm punch) 1–21 d after IL-2 administration, cut into three parts, and processed for evaluation as described below.

**Histopathology and Enumeration of Bacilli.** A part of each biopsy was fixed in 10% neutral buffered formalin overnight, embedded in paraffin, sectioned, and stained for histological examination. Some of the biopsies were stained by the Triff stain for immediate evaluation of histologic changes. The rest of the tissue was transported back to the U. S., 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 (14, 15) was used to express bacterial numbers in control and IL-2-injected sites. Of the 35 patients tested, only 22 had a high enough BI to be used for bacterial reduction analysis.

**Immunohistology.** Biopsy specimens were fixed in paraformaldehyde (3%), lysine (0.075 M), and sodium-*m*-periodate (0.01 M) in PBS (PLP) for 3–4 h at 4°C as described by McLean and Nakane (16). This fixative preserves structural details without inhibiting the binding of mAbs to their antigens. The biopsies were washed in PBS containing sucrose (10%) and digitonin ( $5 \times 10^{-5}$  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. and 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., Burlington, CA). The reaction product was developed with 0.8 mg/ml 3-amino-9-ethylcarbazole and 0.015% H<sub>2</sub>O<sub>2</sub>. Sections were counterstained with hematoxylin.

**Monoclonal and Polyclonal Antibodies.** Mouse mAbs were used for the identification of specific cell types. Leu-1, Leu-2a, and Leu-3a (17, 18) (CD5, CD4, and CD8 anti-T cells) and Leu-M5 (CD11c anti-monocyte/macrophage) (19) were obtained from Becton Dickinson & Co. (Mountain View, CA). OKT6 (20) (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 (21). Rabbit anti-γ IP-10 antibodies were produced in this laboratory (22).

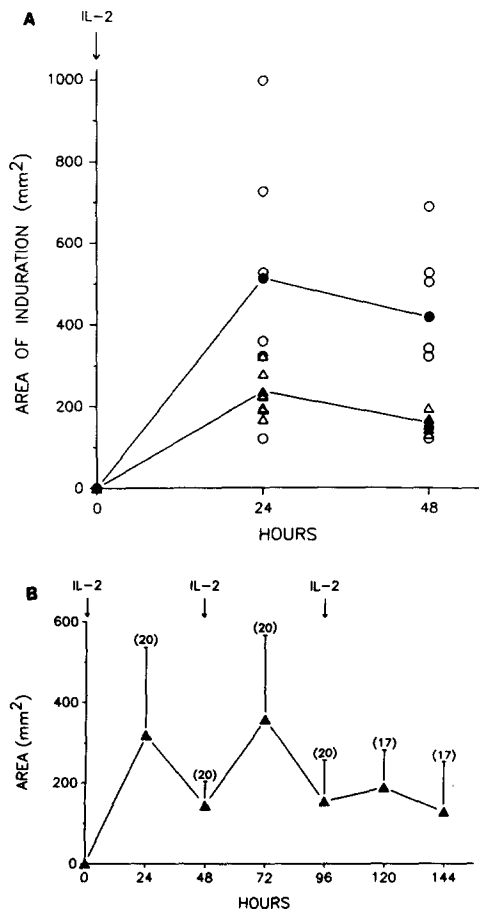
**Determination of Epidermal Thickness.** Epidermal thickness was evaluated by direct examination of hematoxylin-stained sections using a computer-based image digitizing system

(Southern Micro Instruments, Inc., Atlanta, GA). The distance from the epidermal-dermal junction to the outer surface of the epidermis was measured at equal intervals along the length of the sections. At least 25 measurements were made per section. Results were expressed as the ratio of mean thickness of the IL-2-injected site vs. the control site for each patient.

**EM.** A part of each biopsy was processed for transmission EM studies. Biopsies were washed in saline at 4°C, cut into 1–2-mm pieces, and 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. The tissues were cut to 1 mm or smaller and postfixed in 2% OsO<sub>4</sub> for 6 h at 4°C. The tissue was then stained en block for 2 h with 0.25% uranyl acetate, dehydrated in increments with ethanol, and embedded in epon blocks. Semi-thin sections were stained with methylene blue-azur-basic fuchsin and examined for areas containing infiltrating cells. Sections were stained with uranyl acetate and lead citrate, and examined with a transmission EM (model JEM 100CX; Jeol USA, Peabody, MA). At least 200 cells from each patient were examined and photographed on Kodak electron image film.

**Results**

*Local IL-2 Responsiveness of Lepromatous Patients.* The local reaction to the intradermal injection of a single dose of 10 or 25 µg of rIL-2 into apparently normal skin of lepromatous leprosy patients is shown in Fig. 1 A. All the injected patients responded



**FIGURE 1.** Local induration in response to rIL-2 administration. (A) The area of induration in response to a single intradermal injection of 10 µg (Δ) or 25 µg (○) of rIL-2. The mean response for all the patients tested is shown by the closed symbols. Induration was high at 24 h and reduced at 48 h. (B) The area of induration in response to two or three intradermal injections of 10 µg rIL-2 administered at 48-h intervals. Results are means of induration. The bars denote 1 SD and the numbers in parenthesis the number of individuals tested. Induration was high 24 h after the first and second injection and subsided to a lower based levels that persisted. The arrows mark the time of IL-2 injection.

to the injection of IL-2. The extent of induration (radius of indurated area) was proportional to the dose of lymphokine administered both at 24 and 48 h after injection. Induration peaked at 24 h and decreased at 48 h, to a level that persisted for 4-7 d (not shown). This response is comparable with a strong delayed-type hypersensitivity (DTH) response, but DTH usually persists for at least 96 h without any decrease (10).

The local response to multiple doses of 10  $\mu$ g IL-2 given at 48-h intervals is shown in Fig. 1 B. As in the previous group of patients, the initial induration after the first, second, and third injection (24, 72, and 120 h) was reduced 24 h later at 48, 96, and 144 h, respectively. The area of induration induced by the first injection was not enhanced by subsequent injections. On the contrary, the third injection did not lead to induration above the basal level observed at 48 and 96 h. This suggested an unresponsiveness to IL-2 at 96 h. The basal induration observed at 96 and 144 h persisted for up to 2 wk. By 3 wk after lymphokine administration, 8-10 mm of induration was still observed in about half of the patients biopsied at that time (not shown). Other than an erythematous skirt about the 24-h site, no other local or systemic reactions were noted.

*The Local Cellular Response to rIL-2 Administration.* Histological and immunohistological sections of IL-2-injected and control sites biopsied 2-21 d after lymphokine administration were used for the evaluation of the local cellular response to lym-

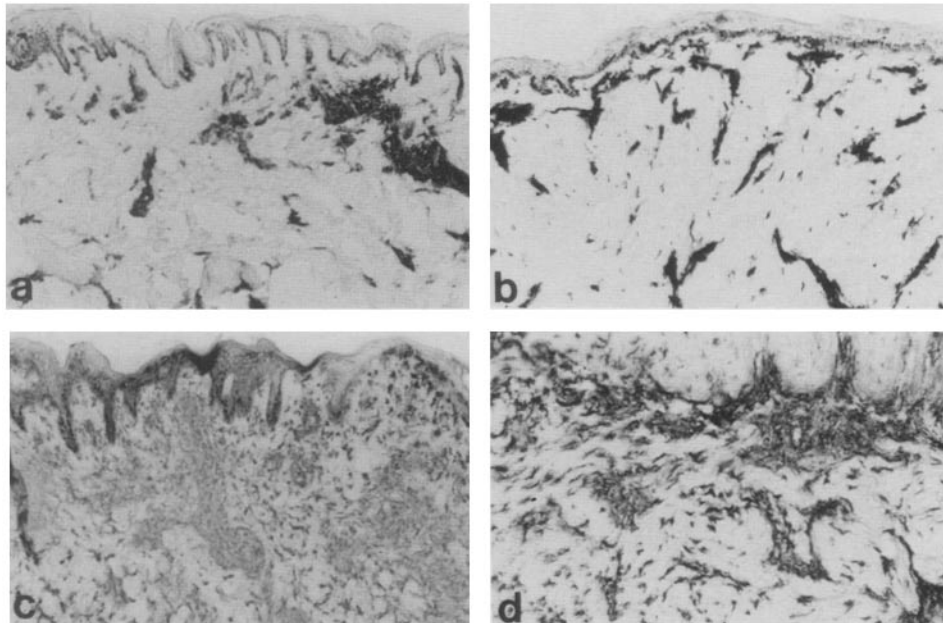


FIGURE 2. The extent of dermal infiltration in response to rIL-2. Immunohistologic staining of cryostat sections with anti-Ia. Biopsies were taken from a patient (Table I, patient 4) at time 0 (a) and at 48 h after a single 10- $\mu$ g rIL-2 injection (c) and from another patient (Table I, patient 17) at time 0 (b) and 21 d after three 10- $\mu$ g injections of rIL-2 (d). Note the extensive staining of cells in the dermis, the thickening of the epidermis, and the expression of Ia on the surface of the keratinocytes after IL-2 administration ( $\times 50$ ).

phokine. The control sites taken from nonlesional "normal" skin contained an inflammatory infiltrate of lepromatous leprosy with perivascular aggregates of mononuclear leukocytes involving from <5–30% of the dermis (Fig. 2, *a* and *b* and Table I). Control biopsies taken from macular or nodular lepromatous lesions demonstrated dermal infiltration of 5–95% (Table I). The injection of rIL-2 into the skin induced an early extensive recruitment of new mononuclear cells that enhanced the percent of the dermis involved (Fig. 2, *c* and *d*). The enhancement in cellularity was evaluated and expressed as the ratio of the area of dermis containing inflammatory cells in the IL-2 vs. the control site. As illustrated in Fig. 3 *A*, the increase in dermal, cellular infiltration was well established by 48 h, peaked at 4 d, and then subsided. By 3 wk the percent of the dermis involved at the IL-2 site had returned to the control level in the majority of patients.

The steep decline in cellularity after the last injection of IL-2 suggested that the lymphokine could be changing the normal turnover and or recirculation of the lym-

TABLE I  
*Influence of IL-2 on the Bacterial Indices of Lepromatous Leprosy Lesions*

Patient	Dose IL-2	Time biopsy	Percent infiltration		Diagnosis*		Bacterial index		Reduction in BI <sup>‡</sup>	Granuloma post-IL-2 <sup>§</sup>
			Control	IL-2	Control	IL-2	Control	IL-2		
		<i>µg</i>								
1	10	48 h	<5	20	LL	LL subp <sup>  </sup>	6+	<6+	±	-
2	10	48 h	70 <sup>¶</sup>	30	LL	BL	6+	4-5+	2-1+	-
3	10	72 h	30 <sup>¶</sup>	40	LL	LL subp <sup>  </sup>	6+	<6+	±	-
4	10	72 h	10	30	LL	BL	5+	3-5+	2-0+	-
5	25	72 h	<5	25	BL	BL	4+	3+	1+	-
6	25	72 h	<5	10	BL	BL	3+	5+	Negative	-
7	25	72 h	<5	40	BL	BL	5+	4+	1+	-
8	2 × 10	96 h	<5	45	BL	LL	5+	3+	2+	-
9	3 × 10	7 d	50**	80	LL	LL	6+	<6+	±	-
10	3 × 10	7 d	40	70	LL	BL	6+	5+	1+	-
11	3 × 10	12 d	80**	70	BL	BL	3+	1+	2+	+
12	3 × 10	13 d	5	15	BL	BT	3+	1+	2+	+
13	3 × 10	13 d	15	25	BL	BT	4+	1+	3+	+
14	3 × 10	19 d	40**	80	LL	LL	5+	5+	0	+
15	3 × 10	19 d	30**	50	LL	BL	6+	5+	1+	+
16	3 × 10	20 d	70**	95	LL	LL	6+	<6+	±	+
17	3 × 10	21 d	50**	25	LL	BL	6+	4+	2+	+
18	3 × 10	21 d	10	20	BL	BL	2+	1+	1+	-
19	3 × 10	21 d	80**	70	BL	BL	4+	4+	0	+
20	3 × 10	21 d	40**	70	LL	LL	5+	4+	1+	+
21	3 × 10	21 d	95**	90	LL	BL	6+	4+	2+	-
22	3 × 10	21 d	70**	70	LL	LL	6+	<6+	±	+

<6+, patchy reduction, some areas 6+, others 5+ or less.

\* The diagnosis was the histologic diagnosis of the control and IL-2 injected biopsies according to the Ridley Jopling scale.

‡ The reduction in BI is expressed in BI units (log units) and is the difference between the control and the IL-2 BI in the adjacent columns. ±, <10-fold.

§ Granulomata were defined by the presence of epithelioid and multinucleated giant cells.

|| Subpolar lepromatous leprosy.

¶ IL-2 injected into "normal skin" between nodular lesions.

\*\* IL-2 injected into nodular lesions. All other patients injected into "normal skin" or macular lesions.

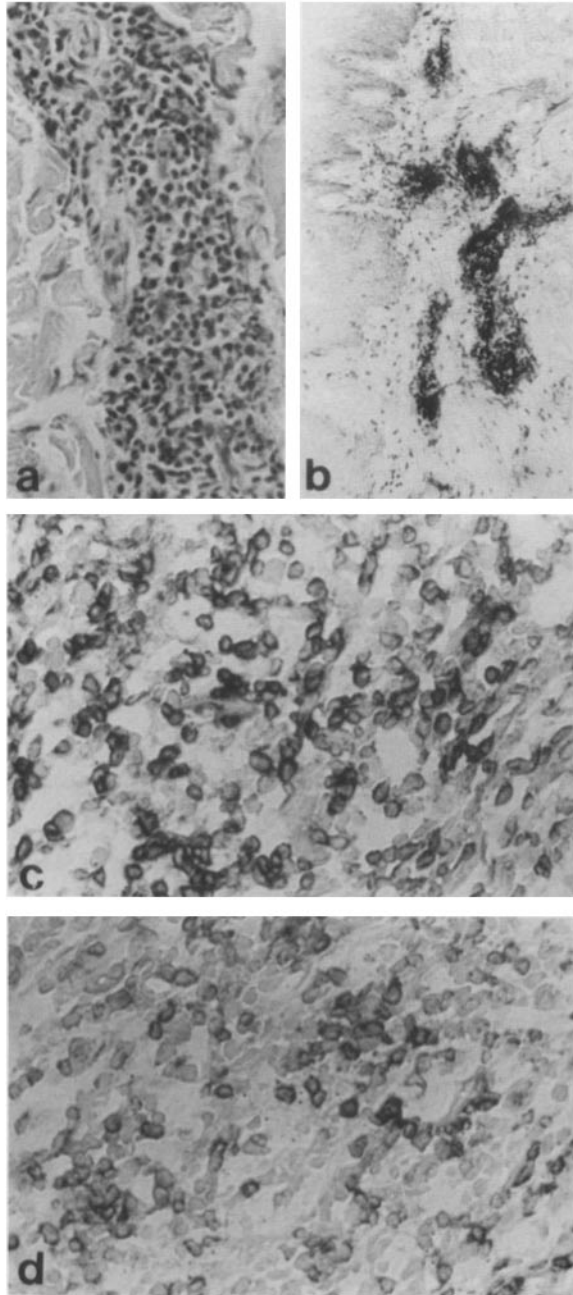


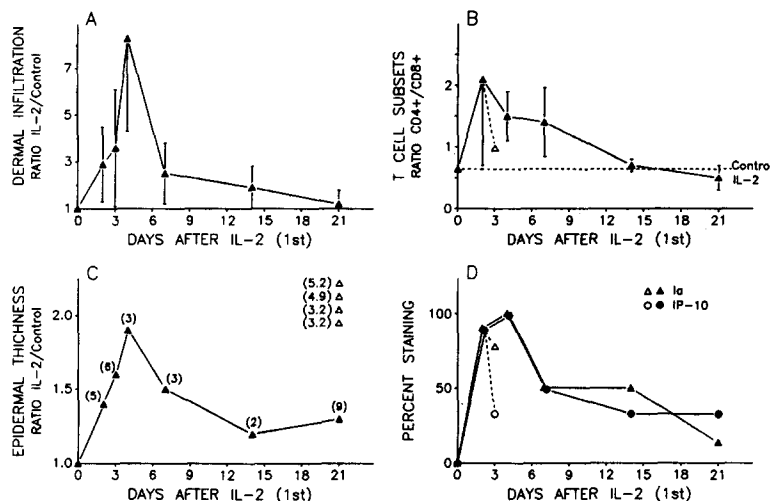
FIGURE 3. T cell accumulation and subset distribution during a IL-2 response. Histologic (Hematoxylin and eosin) (a) and immunohistologic staining of sections with mAbs that recognize all T cells (CD5) (Leu-1) (b and c) and the CD8<sup>+</sup> subset of T cells (Leu-2a) (d). Biopsies were taken 96 h after IL-2 injections ( $2 \times 10 \mu\text{g}$ ). At this early time the majority of T cells are of the CD4<sup>+</sup> subset ( $\times 100$  [a];  $\times 50$  [b]; and  $\times 200$  [c and d]).

phocytes through the lesion. It is possible that new cells did not continue to enter the local site in the absence of additional lymphokine administration. Instead, many of the cells either left the dermis and/or were killed and cleared locally within a day or two. This observation was corroborated by histological sections of the sites biop-

sied 48–96 h after IL-2 injection, which revealed extensive cell damage and many pyknotic nuclei. The lesions did not appear necrotic but showed selective death and clearance of some cells intermixed with intact, viable cells (see below).

**T Cells and Their Subsets.** The new inflammatory cells recruited by IL-2 could be identified as T lymphocytes and monocytes by their surface phenotype (Fig. 3). At 48 h, the earliest time point examined in detail, the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells was over three times that observed in the control lepromatous skin and lesions (2:1 vs. 0.6:1) (Fig. 4 B). In the absence of additional IL-2 administration, this ratio returned to control levels by the next day (Fig. 4 B, open triangle). Repeated injections of rIL-2 maintained both a high number as well as a high ratio of CD4<sup>+</sup>/CD8<sup>+</sup> cells. By 2 wk the ratio had returned to control levels in all patients while total cellularity was still above that found in the matched control sites. This could be the result of retention of mononuclear phagocytes and T cells and/or limited continued emigration.

**Effect of IL-2 Administration on the Epidermis.** Microscopic examination of the epidermis overlying the IL-2 sites revealed extensive epidermal thickening as compared with the matched control sites in all patients biopsied 2–4 d after IL-2 injection (Fig. 2). The kinetics of thickening corresponded with the accumulation of cells in the dermis (Fig. 4 C). By 7 d a reduction in the thickness of the IL-2-injected



**FIGURE 4.** Kinetics of the local response to intradermal administration of rIL-2. (A) Enhancement of the percent of the dermis infiltrated by mononuclear cells in response to IL-2. Results are expressed as the ratio of the percent of area of the dermis infiltrated in the IL-2 injected site vs. the control site for each patient. (B) T cell subsets expressed as CD4<sup>+</sup>/CD8<sup>+</sup> ratio in the rIL-2 injected and control sites. (C) Epidermal thickening in response to IL-2 was expressed as ratio of thickness in the IL-2 injected vs. the control site of each patient. The results for four individuals (open symbols) evaluated at 21 d deviated from the majority and are shown separately as the value in parenthesis. (D) Expression of MHC class II (Ia) and IFN- $\gamma$ -induced peptide (IP-10) on keratinocytes. Results expressed as the percent of individuals with positive staining of the keratinocytes in the epidermis after IL-2 injection. All results were evaluated 1–21 d after the first rIL-2 injection. The results are means (A–C)  $\pm$  1 SD (A and B). The numbers in parenthesis (C) are the number of individuals tested. The open symbols (B and D) denote the results 48 h after a single injection of 10  $\mu$ g rIL-2.

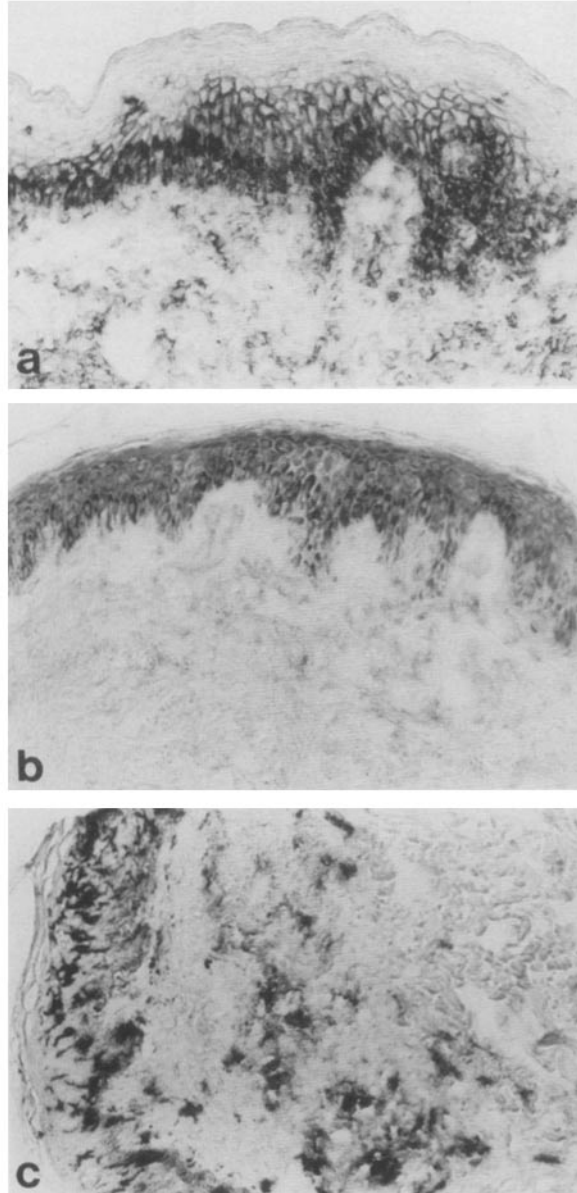


FIGURE 5. Ia and IP-10 expression on Keratinocytes and Langerhans' cell distribution after IL-2. Immunohistologic staining of sections with mAbs to MHC class II (9.3) (a), IFN- $\gamma$ -induced IP-10 (b), and CD1<sup>+</sup> Langerhans' cells (OKT6) (c). Biopsies were taken 96 h after two doses of 10  $\mu$ g IL-2 (a), 48 h after 10  $\mu$ g IL-2 (b), and 21 d after  $3 \times 10$   $\mu$ g IL-2 (c). Biopsies taken from uninjected control sites did not stain for Ia or IP-10 (not shown) ( $\times 200$ ).

site was observed. 3 wk after IL-2 administration the epidermis overlying the test site was almost normal in thickness in 9 of 13 patients tested at this time point.

Associated with the thickening of the epidermis were changes in the phenotype of the keratinocytes overlying these lesions. MHC class II antigen (Ia) and the IFN- $\gamma$ -induced peptide IP-10 were expressed on keratinocytes after IL-2 (Figs. 4 D and 5, a and b). Keratinocytes of control biopsies did not express these antigens (not shown). Only 30–40% of individuals tested still expressed Ia and IP-10 on their ker-



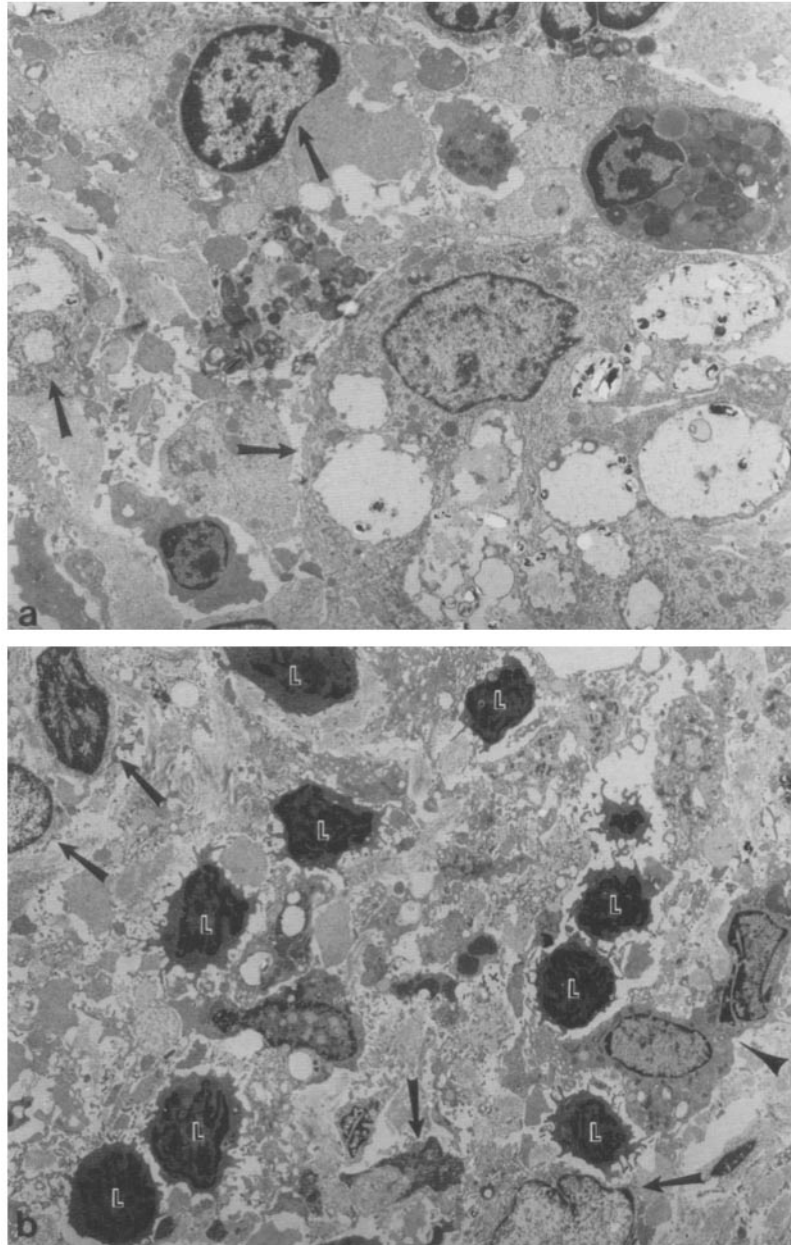


FIGURE 6. EM micrographs of the selective destruction of parasitized foam macrophages. (a) IL-2-injected site ( $3 \times 10 \mu\text{g}$ ) biopsied 7 d after lymphokine administration. The parasitized macrophages are disintegrating or damaged (*arrows*) ( $\times 5,000$ ). (b) Dead and damaged macrophages (*arrowheads*) in association with viable multinucleated phagocytes (*arrow*) and many lymphocytes (*L*) ( $\times 3,000$ ).

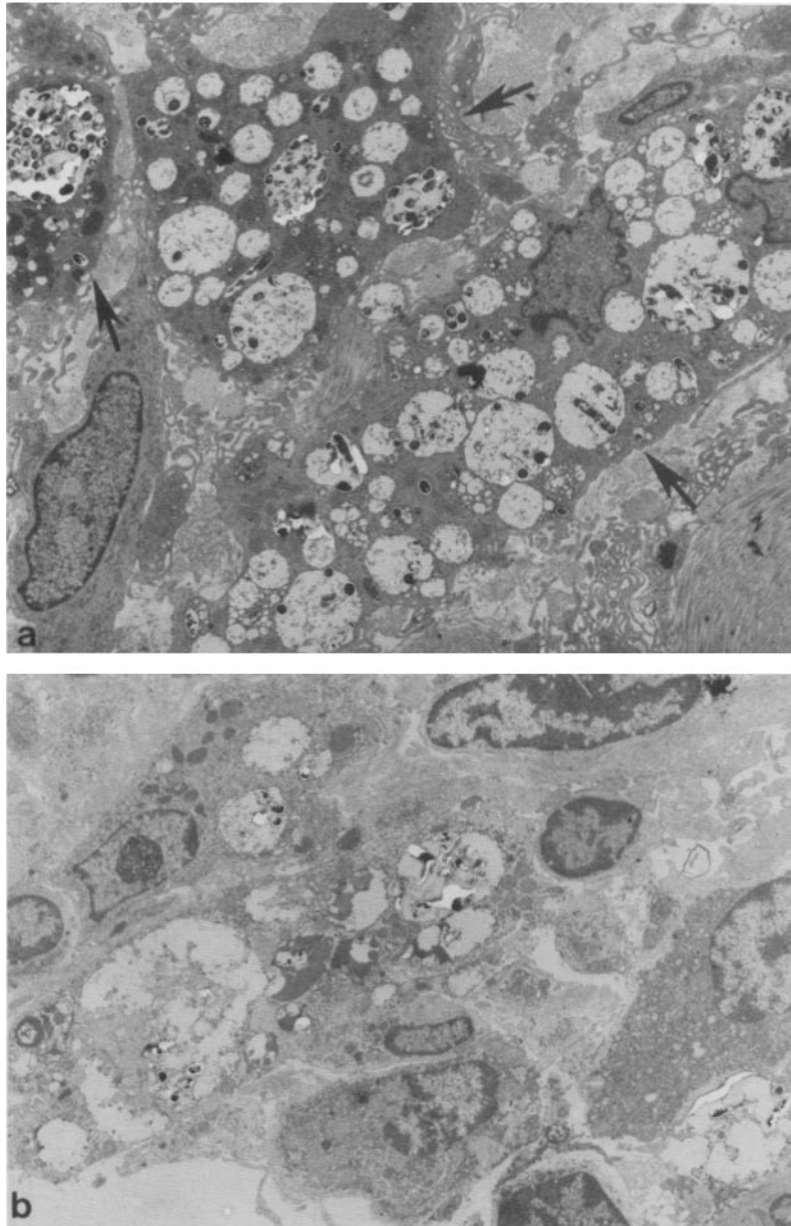


FIGURE 7. Local *M. leprae* clearance in response to IL-2 injection. (a) Large numbers of bacilli, many of them intact, in the vacuoles of three macrophages (arrows) from a control lesion. (b) Reduced numbers of *M. leprae* are seen in the vacuoles of macrophages 21 d after the administration of 3 10- $\mu$ g doses of rIL-2 into a matched lesion (same patient as a) ( $\times$  3,000).

atinocytes at the site of IL-2 injection by 3 wk. Since Ia and IP-10 are both IFN- $\gamma$ -responsiveness genes, these results are in keeping with the local release of IFN- $\gamma$ .

*Effect of IL-2 Administration on the Distribution of T6<sup>+</sup> Langerhans' Cells.* In control lepromatous leprosy lesions, T6<sup>+</sup> Langerhans' cells were evenly distributed in the epidermis and absent from the dermis (23). After IL-2 administration T6<sup>+</sup> LC were prominent in the dermis by 72 h and persisted for as long as 3 wk (Fig. 5 c). No modification in Langerhans' cell number or distribution was noted in the overlying epidermis as reported after PPD administration (23).

*Mononuclear Phagocyte Differentiation and the Selective Destruction of Parasitized Macrophages.* After the IL-2-induced entry of new monocytes into the dermis, the cells differentiated into epithelioid and multinucleated giant cells (Fig. 6 b). Epithelioid cell granulomas were observed 12 d after multiple injections of lymphokine and persisted for at least 3 wk (Table I). The differentiated mononuclear phagocytes of the granulomata were observed in association with lymphoid T cells of both the CD4<sup>+</sup> and CD8<sup>+</sup> phenotype.

Transmission EM revealed extensive local destruction of parasitized macrophages and foam cells in association with the accumulation of lymphoid cells in the IL-2-injected sites (Fig. 6 a). Cellular dissolution and extracellular discharge of cell organelles, phagolysomes containing *M. leprae*, and free bacteria was prominent as early as 48 h after IL-2 injection.

*Local M. leprae Clearance.* Associated with the destruction of parasitized and foamy macrophages, there was a reduction in the number of *M. leprae* of the lesion (Fig. 7 and Table I). A patchy reduction of up to 10-fold in *M. leprae* numbers was observed in six of seven patients biopsied as early as 48–72 h after IL-2 injection (Table I). In patients biopsied 4–21 d after IL-2 injection, a reduction of 10–1,000-fold in bacterial numbers was observed in 13 of 15 patients tested. This occurred both in nodular lesions as well as macular and diffuse lesions of "normal skin," and was associated with up-grading of the Ridley-Jopling diagnostic index.

## Discussion

We have evaluated the cellular and microbial responses in the skin of LL/BL patients injected intradermally with 10–30  $\mu$ g doses of rIL-2. In doing so, we have recapitulated the essential features of a cell-mediated immune response, previously established for an antigen-driven reaction to PPD (9, 10, 22–24). These include extensive and persistent emigration of T cells and monocytes, the preponderance of T cells with a high CD4<sup>+</sup>/CD8<sup>+</sup> ratio, the dermal accumulation of T6<sup>+</sup> Langerhans' cells, the differentiation of mononuclear phagocytes to form granulomata, induction of expression of keratinocyte Ia and IP-10, and epidermal thickening.

In association with these events there is a striking, selective destruction of parasitized macrophages. We feel this destruction is an essential element in the subsequent disposal of *M. leprae*. We consider the resident infected dermal macrophage defective in those agents that can effectively kill and digest *M. leprae* within the phagolysosome (25, 26). To expose these organisms to a less favorable intracellular milieu, one mechanism would involve the extracellular liberation of the bacteria and their subsequent uptake by newly emigrated, oxidatively competent monocytes (25–28). Prior in vitro studies indicate that blood-borne monocytes are responsive to the IFN- $\gamma$  produced (29) by CD4<sup>+</sup> and CD8<sup>+</sup> T cells, NK, and LAK cells. All these cell types are likely

candidates for local production of the lymphokine in the dermal site. Thus, working in concert, macrophage-activating lymphokines and newly emigrated monocytes are effective in the killing and degradation of viable *M. leprae*.

Our evidence for killing and degradation of *M. leprae* comes largely from quantitative morphological studies conducted at the light and electron microscopic level. The significant and consistent decline in local microbial load is quite remarkable when one considers the natural history of the disease. Even after microbicidal chemotherapy, the decline in nonviable organisms in the skin occurs at a rate of only 1.0 BI units per year (30). In contrast, IL-2 and the cellular response it evokes leads to the local disposal of 2–3 BI units in 3 wk or an increased removal of bacilli that exceeds the baseline rate by a factor of 20–200. The response to IL-2 is more rapid and extensive than that observed either with PPD or rIFN- $\gamma$  (9, 10). Reductions in organisms occur within 1 wk of IL-2 injection and are as evident in the nodular lesions of LL/BL patients and in so-called “normal” skin. rIFN- $\gamma$  modifies the bacillary load of nodular leprosy at a much slower rate (Kaplan et al., manuscript in preparation).

The requirement for the selective destruction of parasitized macrophages is of considerable interest and may well apply to other situations in which bacteria or protozoal parasites lead to chronic infection. We have evidence that this occurs with the infected Kupffer cells of hepatic granuloma in murine Leishmanial infections (31) and in the local destruction of *Leishmania aethiopica*-infected macrophages in chronic disseminated leishmaniasis in man after IL-2 administration (manuscript in preparation). Observations made previously by us indicate that a similar scenario takes place in the natural lesions of paucibacillary tuberculoid leprosy and after the intradermal injection of PPD in presensitized lepromatous patients. In each of these instances, T cells have been observed in close contact with dead and dying epithelioid and foamy macrophages containing bacilli and bacillary products. Additional insights are now coming from the in vitro experiments of Hancock et al. (32), which demonstrate the ability of CD4<sup>+</sup> T cells to kill antigen (PPD)-pulsed macrophages in an antigen-dependent, MHC class II antigen-restricted way. Since many lepromatous patients fail to respond to specific *M. leprae* antigens, we suspect that crossreactive epitopes play a major role in the recognition of the parasitized macrophages in this form of toxicity. In addition, both NK cells and lymphokine-activated killer cells can play a less-specific role in macrophage destruction. Our in vitro studies suggest that PPD stimulation as well as exposure to rIL-2 generate an antigen-independent cytotoxic effector population that can kill monocytes in vitro.

The dermal induration and cell infiltration in response to IL-2 is briefer but more uniform than observed with antigens such as PPD. All patients respond to the lymphokine and the unresponsiveness to *M. leprae* does not appear to affect the responsiveness of IL-2. The doses used in this study are miniscule when compared with the experience with antitumor therapy (33) but evoke both a local and systemic T cell response without untoward symptomatology. They are high relative to the amounts of IL-2 required for cellular activation in vitro (11, 12). In studies to be reported elsewhere, PBMC from these patients showed more extensive proliferation (TdR incorporation) to antigens to which they have been previously sensitized after IL-2 injection. Obviously, many other questions remain concerning the effects of IL-2 when injected into the skin. Is it the primary or secondary chemoattractant that

leads to extensive mononuclear cell emigration? What is the nature, source, and amount of induced molecules synthesized by activated cells in response to IL-2 administration? These questions are currently under investigation.

Finally, the use of IL-2 as a therapeutic agent requires additional information concerning its potential use for parenteral therapy. Studies in mice have shown that IL-2 administered subcutaneously can protect the animals against infections and enhance their immunity (34–36). We do not know whether, if used parenterally, IL-2 could enter and modify the dermis of LL/BL patients or whether local modifications of endothelial permeability would be required. Parenteral IL-2 has been shown to activate the endothelium, enhance vascular permeability, and induce dermatological changes, including migration of T cells into the skin (37, 38). These are questions for the future but ones that are pertinent for a wide range of intracellular parasites.

### Summary

Human rIL-2 (10–30  $\mu\text{g}$ ) was injected intradermally into the skin of patients with lepromatous leprosy with high bacillary loads. All patients responded to the lymphokine with local areas of induration that peaked at 24 h and persisted for 4–7 d irrespective of whether the site was “normal skin” or a nodular lesion. Within 24 h there was an extensive emigration of T cells and monocytes into the site. The percentage of the dermis infiltrated by mononuclear cells increased by more than sevenfold, peaking at 4 d and persisting for >15 d. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells entered the site. T cells of CD4<sup>+</sup> phenotype predominated at 2–7 d but by 11 d, CD8<sup>+</sup> cells were predominant. Considerable numbers of T6<sup>+</sup> Langerhans’ cells appeared in the dermis by 72 h and persisted for 3 wk. By 4 d the thickness of the overlying epidermis had increased twofold, and keratinocytes were expressing MHC class II antigen and the IFN- $\gamma$ -induced peptide IP-10.

Starting at 48 h, there was an extensive destruction of mononuclear phagocytes that contained structurally intact or fragmented *M. leprae* observed at the electron microscope level. The organisms, either free or contained within endocytic vacuoles, were discharged into the extracellular space and then reingested by blood-borne monocytes. This was followed by marked reductions in the number of acid-fast organisms in the injected site, evident as early as 4–7 d and more marked at 2–3 wk after injection. 13 of 15 patients exhibited a disposal of acid-fast bacilli ranging from 5- to 1,000-fold with a mean value of  $\sim$ 100-fold.

The administration of IL-2 leads to the generation of an effective cell-mediated immune response, recapitulating an antigen-driven event and leading to striking local reductions in *M. leprae*. In comparison with the purified protein derivative of tuberculin reaction, bacilli are cleared more promptly, although emigratory cells persist for a shorter time.

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