

## *In Vivo* Responses to *Mycobacterium leprae*: Antigen Presentation, Interleukin-2 Production, and Immune Cell Phenotypes in Naturally Occurring Leprosy Lesions<sup>1,3</sup>

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The central feature of the lepromatous forms of leprosy, those forms with low host resistance, is ineffective killing of *Mycobacterium leprae* by histiocytes. No specific differences between lepromatous macrophages and normal macrophages have been demonstrated *in vitro* (<sup>6, 26</sup>), and research has concentrated on T lymphocytes which influence macrophage function. Cells of the OKT8-Leu2 (suppressor/cytotoxic) phenotype are the predominant lymphocyte subtype in lepromatous lesions. Whether they are the cause of the defective response to *M. leprae* by lepromatous patients is questionable, since they are present in lesions of borderline and tuberculoid patients as well as lepromatous patients (<sup>14, 16, 27</sup>). Moreover, *M. leprae* antigen-specific suppression has been observed in peripheral blood mononuclear cells from healthy contacts of leprosy cases (<sup>12, 25</sup>) as well as patients throughout the spectrum, indicating that antigen-specific suppressor cells possibly

play a role in the normal effective response to *M. leprae*.

Perhaps more relevant to the actual destruction of bacilli is the relative paucity of T cells with the OKT4-Leu3 (helper/inducer) phenotype in lepromatous lesions. These cells produce lymphokines including gamma interferon, a known macrophage activator (<sup>17</sup>). Recent *in vitro* studies have shown that peripheral blood mononuclear cells from at least some lepromatous leprosy patients are able to react to *M. leprae* antigens up to the point of interleukin-2 (IL-2)-induced proliferation, and that they will proliferate and produce gamma interferon (<sup>18</sup>) if exogenous IL-2 is added to the culture system (<sup>8, 18</sup>). To investigate the hypothesis that the lack of IL-2-induced T-cell proliferation with resultant absence of effective macrophage activation is central to immune system failure in lepromatous leprosy, we examined populations of cells in leprosy lesions to determine if the phenotypes associated with various functions of the immune cascade are present.

### MATERIALS AND METHODS

**Study population.** Seventeen patients attending the All Africa Leprosy Rehabilitation and Training Centre (ALERT) Hospital in Addis Ababa, Ethiopia, were selected randomly. All were native Ethiopians. Sixteen were untreated patients with nonreactive leprosy attending the diagnostic clinic. One patient who was being treated with dapsone but who had primary infection with dapsone-resistant organisms was also included. Patients were classified clinically and histologically according to the Ridley-Jop-

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TABLE 1. *Characteristics of patients.*

Patient	Sex	Age	Classification	
			Clinical	Histo-logical
J58	F	23	TT	TT
222/83	M	50	TT	TT
158/83	M	15	TT	TT
421/83	F	46	TT	BT
328/83	M	70	TT-BT	BT
700/83	F	29	BT	BT
701/83	F	35	BT	BT
760/83	M	35	BT	BT
329/83	F	19	BB	BB
686/83	F	19	BL	BL
743/83	M	17	BL	BL
773/83	M	34	BL	BL
704/83	M	16	BL	BL
629/83	M	32	LL	LL
687/83	F	16	LL	LL
777/83	M	38	LL	LL
798/83	M	37	LL	LL

ling scale (<sup>23</sup>) (Table 1). Samples of peripheral blood were drawn at the time of biopsy.

**Biopsy analysis.** Biopsies were taken with a standard, dermatologic biopsy punch. Each biopsy was divided into two halves. One half was used for routine histological diagnosis, including determination of the percentage of the dermis infiltrated by granuloma, and the other half was placed in an airtight plastic capsule, covered with optional cutting temperature (OCT) compound (Miles Laboratories Inc., Elkhart, Indiana, U.S.A.), snap frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$  until used. Six  $\mu\text{m}$  thick serial sections were cut onto microscope slides on an Ames Cryostat II, fixed for 5 sec in acetone at  $4^{\circ}\text{C}$ , and air dried overnight at  $-20^{\circ}\text{C}$ . Single immunohistochemical staining was done using a modification of the avidin-biotin horseradish peroxidase complex method (Vector Laboratories, Burlingame, California, U.S.A.) (<sup>9</sup>). Briefly, sections were fixed in acetone at  $4^{\circ}\text{C}$  for 10 min ( $27^{\circ}\text{C}$  for cytoplasmic staining of IL-2), air dried for 2 min, washed in phosphate buffered saline (PBS), and incubated sequentially with the following reagents: normal horse serum, mouse hybridoma monoclonal antibody, biotinylated horse anti-mouse-IgG serum,  $0.3\%$   $\text{H}_2\text{O}_2$  in methanol, and avidin-biotin horseradish peroxidase complex. The reaction product was developed by incubating

in  $0.1\%$  diaminobenzidine and  $0.3\%$   $\text{H}_2\text{O}_2$  in PBS, a  $0.9\%$  saline wash, followed by  $1\%$   $\text{CuSO}_4$  in  $0.9\%$  saline. Sections were counterstained with methylene blue, toluidine blue, or light green solutions, dehydrated and mounted in Permount (Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.).

Double staining was done on tissue prepared and processed identically except that the primary mouse hybridoma monoclonal antibody could not be of the IgG<sub>1</sub> subtype. After the incubation with biotinylated horse anti-mouse IgG, the following reagents were used sequentially, alternating with PBS washes unless otherwise stated: a repeat incubation with primary antibody, a second mouse hybridoma antibody of the IgG<sub>1</sub> subtype,  $0.3\%$   $\text{H}_2\text{O}_2$  in methanol, avidin-alkaline phosphatase conjugate (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) in  $0.1\text{ N}$  bicarbonate ( $\text{pH } 9.4$ ) (<sup>3</sup>),  $10\%$  normal sheep serum, affinity purified sheep anti-mouse IgG<sub>1</sub> conjugated to horseradish peroxidase (Serotec, Oxford, England), DAB substrate,  $0.05\%$  Tris buffered  $0.9\%$  NaCl (no more PBS washes), alkaline phosphatase substrate ( $0.5\text{ mg/ml}$  naphthal AS-MX phosphate,  $1\text{ mg/ml}$  fast blue BB salt,  $2\text{ mM}$  levamisole HCl in  $0.2\text{ M}$  Tris,  $\text{pH } 8.2$ ) (<sup>11, 19</sup>), tap water wash,  $0.9\%$  NaCl,  $1\%$   $\text{CuSO}_4$  in  $0.9\%$  NaCl followed by a tap water wash. Some slides were counterstained with nuclear-fast red. Slides were mounted in gelvatol. Cell counts were made of representative portions of specimen granulomas using an American Optical microscope with a grid eyepiece. Five hundred to 1000 stained cells were counted in each specimen when available and cell counts adjusted to  $1\text{ mm}^2$  of the granuloma. When infiltrates were small, all stained cells in the section were counted.

**Peripheral blood studies.** Peripheral blood mononuclear cell populations were enumerated in suspension using mouse monoclonal hybridoma primary antibodies and indirect immunofluorescence techniques as previously described (<sup>7</sup>).

**Monoclonal antibodies.** Antibody titers employed for the study were determined by multiple dilutions and staining of several specimens. Titers are reported in parentheses following the antibody type. Primary mouse hybridoma monoclonal antibodies reacting with human antigens were gener-

TABLE 2. Naturally occurring leprosy lesions.

Group	No. <sup>a</sup>	OKT8 <sup>b</sup>	OKT4-Leu3 <sup>b</sup>
TT-BT	8	900 ± 296	1412 ± 288
BB	1	1175	1293
BL	4	1337 ± 225	400 ± 93 <sup>c</sup>
LL	4	787 ± 325	200 ± 100 <sup>c</sup>

<sup>a</sup> Number of cases examined.

<sup>b</sup> Numbers in columns represent number of cells bearing the OKT4-Leu3 or OKT8 phenotype/mm<sup>2</sup> of granuloma in 6 μm thick sections.

<sup>c</sup> Significantly different from mean number of OKT4-Leu3+ cells in the TT-BT group by the *t* test, *p* < 0.001.

ously provided as follows: anti-Tac (1:1500), reacting with the IL-2 receptor complex, from Dr. T. A. Waldman, National Cancer Institute, Bethesda, Maryland, U.S.A. (<sup>4</sup>); DMS-1 (5 μg/ml) reacting with unbound IL-2, Dr. K. A. Smith (<sup>24</sup>); OKT8 (1:40), reacting with the suppressor/cytotoxic T lymphocyte subset; OKT4 (1:25), reacting with the helper/inducer T lymphocyte subset; OKT6 (1:50), reacting with Langerhans' cells; OKB2 (1:20), reacting with surface immunoglobulin-positive B cells; OKM1 (1:100), reacting with monocytes, platelets and granulocytes; OKM5 (1:100), reacting with a macrophage subset including antigen-presenting cells (<sup>5,19</sup>), from Dr. G. Goldstein, Ortho Pharmaceuticals, Raritan, New Jersey, U.S.A.; Leu1 (1:40), reacting with T cells, Dr. C. Y. Wang, Memorial-Sloan Kettering, New York, New York, U.S.A. (<sup>10</sup>), and D4-22 and D1-12 (1:2000), reacting with a nonpolymorphic Ia-like antigen (Class II MHC antigen), Dr. R. S. Accolla, Ludwig Institute for Cancer Research, Lausanne, Switzerland (<sup>1</sup>). Anti-Leu3ab (neat), reacting with the helper/inducer T-lymphocyte subset and weakly with macrophages was purchased from Becton, Dickinson, Mountain View, California, U.S.A.

## RESULTS

**Immune cell phenotypes and IL-2 production in naturally occurring lesions.** Our studies show that cells of the OKT8 phenotype predominate in lesions of polar lepromatous (LL) and borderline lepromatous (BL) patients, confirming findings previously reported by others (<sup>14,16,27</sup>). Our studies on naturally occurring untreated lesions showed the OKT4-Leu3/OKT8 ratio

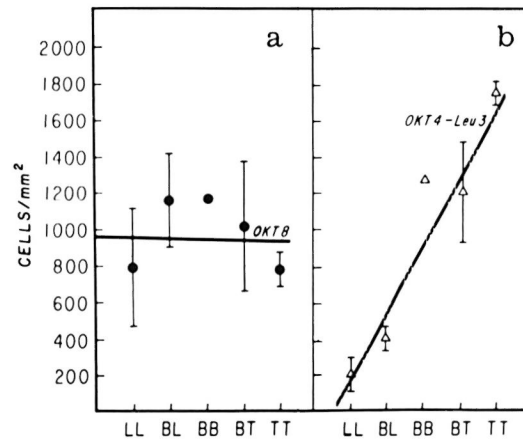


FIG. 1. Shows the relationship between the level of host resistance defined by histologic position on the Ridley-Jopling scale and the phenotypes of lymphocytes present in naturally occurring leprosy skin lesions. The points represent the mean number of a given lymphocyte phenotype/mm<sup>2</sup> of granuloma identifiable in 6-μm-thick frozen sections. Range bars indicate ± one sample standard deviation. Lines indicate the best fit by linear regression. Figure 1a shows the poor correlation between the absolute number of OKT8+ cells/mm<sup>2</sup> granuloma and host resistance (Pearson correlation coefficient -0.02). Figure 1b shows the strong correlation between the absolute number of OKT4-Leu3+ cells/mm<sup>2</sup> granuloma and host resistance (Pearson correlation coefficient 0.95).

to be 0.26 in polar lepromatous lesions, 0.31 in borderline lepromatous lesions, and 1.67 in tuberculoid lesions.

The differences in the lymphocyte subtype ratios across the leprosy spectrum are due primarily to different numbers of OKT4-Leu3 cells (Table 2, Fig. 1). We found comparable numbers of OKT8+ cells per mm<sup>2</sup> in all groups, although the mean number of these cells per mm<sup>2</sup> was slightly lower in the polar groups. The polar lepromatous group had the least number of T lymphocytes (Leu1+ cells/mm<sup>2</sup>) (data not shown). The differences between the mean numbers of OKT4-Leu3+ cells/mm<sup>2</sup> of granuloma counted in 6 μm thick sections in the tuberculoid group (polar tuberculoid or TT and borderline tuberculoid or BT combined, mean 1412 ± 288 cells/mm<sup>2</sup>), the BL group (mean 400 ± 93 cells/mm<sup>2</sup>), and the LL group (mean 200 ± 100 cells/mm<sup>2</sup>) are 1012 and 1212 cells/mm<sup>2</sup>, respectively (Table 2). These differences are significant (*p* < 0.001 by the *t* test). Figure 1 clearly shows a gra-

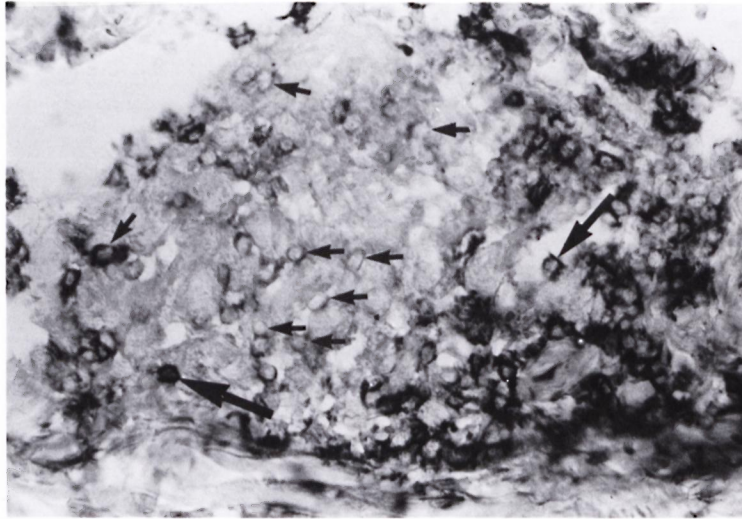


FIG. 2. Photomicrograph of a tuberculoid leprosy granuloma. Original color photomicrograph clearly shows Leu3+ lymphocytes stained as brown rings and present throughout the granuloma. Arrows indicate these cells in the central area of epithelioid differentiation. OKT8+ cells stained dark blue and are confined to the mantle zone, where there are also Leu3+ cells (horseradish peroxidase and alkaline phosphatase  $\times 288$ ).

dient of OKT4-Leu3+ cells as one moves up the spectrum toward the tuberculoid pole. This gradient accounts almost entirely for the gradient of the OKT4-Leu3/OKT8-Leu2 ratio reported by others (<sup>14, 15, 25</sup>). The physiological relationship between the OKT4-Leu3 cells and differentiated epithelioid cells is suggested by the demonstration of segregation of the OKT4-Leu3 and OKT8 phenotypes in tuberculoid granulomas (Fig. 2). This pattern has been inferred previously from staining of serial sections (<sup>8</sup>) but has not been demonstrated in a single section by double staining. Surface immunoglobulin-positive B cells, which we identify by reactivity with OKB2 monoclonal antibody, were rare in all lesions with the exception of scattered positive cells in some BL lesions. The antibody OKM1 and the anti-MCH Class II antibodies D422 and D112 showed diffuse staining of histiocytic cells in lesions throughout the spectrum. This made identification of the MCH Class II antigen expression on individual T cells extremely difficult. OKM5 clearly stained capillary endothelium in all lesions, but the majority of histiocytic cells were negative in all cases.

The absolute numbers of cells bearing the IL-2 receptor/mm<sup>2</sup> (Tac+ cells) were counted and the percentage of Tac+ T cells was

calculated by dividing the number of Tac+ cells/mm<sup>2</sup> by the number of Leu1+ cells/mm<sup>2</sup> and multiplying by 100. The absolute numbers as well as the percentages of T cells bearing the IL-2 receptor were higher in the granulomas of active tuberculoid lesions than in lesions of lepromatous patients (Table 3, Fig. 3). Double staining of several tuberculoid lesions showed that approximately 80% of the Tac+ cells also expressed the Leu3 antigen (Fig. 4a). Insufficient numbers of Tac+ cells were present in lepromatous lesions to accurately determine their phenotype with our techniques. Morphologically, the Tac+ cells ranged from small round cells with dark surface staining present in lesions from all parts of the spectrum to larger cells with lighter surface staining which were found more often in tuberculoid cases. The numbers of OKT6+ cells were greater (mean  $28.2 \pm 3.5/100$  basal nuclei) in the epidermis and dermal granuloma of tuberculoid lesions than in the epidermis and granuloma of lepromatous (LL and BL) lesions (mean  $15.2 \pm 3/100$  basal nuclei) (Table 3). The difference in the numbers of these OKT6+ cells in the granulomas alone was even more marked. There were an average of 8 OKT6+ cells in the granuloma underlying 100 basal nuclei of tuberculoid lesion epidermis, but these cells were rare

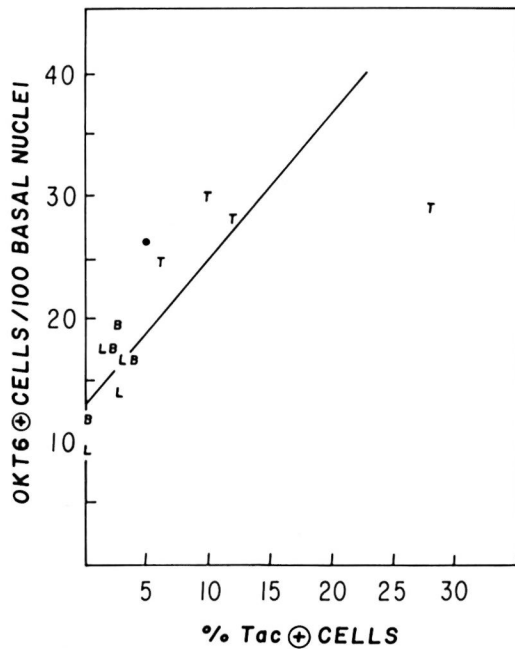


FIG. 3. Correlation between the percentage of Tac+ lymphocytes and the number of OKT6+ cells in the epidermis and in dermal infiltrates per 100 basal nuclei. The values for individual cases are plotted: L = polar lepromatous cases; B = borderline lepromatous cases; T = polar tuberculoid and borderline tuberculoid cases; ● = borderline-borderline cases. The Pearson correlation coefficient is 0.79 ( $p < 0.02$  by the  $t$  test).

in lepromatous granulomas except very superficially at the edges of lesions. Interestingly, we occasionally identified Tac+ lymphocytes in the basal layer of the epidermis where the greatest numbers of OKT6+ cells were seen. The absolute numbers of OKT6+ cells in the epidermis and granuloma were correlated significantly with the percentage of Tac+ cells in the granuloma (Fig. 3). The

number of dendritic cells expressing MCH Class II antigen in the epidermis was similar to the number of OKT6+ cells in the epidermis in individual lesions. The extensive expression of MCH Class II antigens by histiocytes obviated counting dendritic cells in the granulomas with anti-MCH Class II antibodies. This suggests that, at least in the epidermis, antigen-presenting cells do not lose either the OKT6 or MCH Class II antigens in lepromatous leprosy.

Staining for IL-2 was done with the DMS-1 monoclonal antibody. DMS-1 reacts with IL-2 at the site where it binds to the IL-2 receptor. DMS-1 will not bind IL-2 when it is bound to the IL-2 receptor, and it will not displace IL-2 from its receptor because the affinity of DMS-1 for IL-2 is less than that of IL-2 for its receptor (<sup>24</sup>). DMS-1 showed intense staining in a cytoplasmic pattern which was different from the surface staining pattern seen with the IL-2 receptor (Fig. 4b). We interpret this cytoplasmic staining pattern of unbound IL-2 to represent production of IL-2 by the stained cells. IL-2-producing cells were rare in the lesions we examined, but there was a significantly higher percentage of the total lymphocytes (Leu1+ cells) producing IL-2 in tuberculoid lesions than in lepromatous lesions. The difference was greater than one order of magnitude ( $0.032 \pm 0.037\%$  in tuberculoid vs  $0.0019 \pm 0.0023\%$  in lepromatous) (Table 3). Double staining with the OKT6 monoclonal antibody and DMS-1 occasionally showed the close apposition of OKT6+ and IL-2-producing cells in tuberculoid patients, but this was not a constant finding.

**Immune cells in peripheral blood.** The peripheral blood studies showed no significant differences among clinical groups in

TABLE 3. OKT6+, IL-2-producing and IL-2-receptor+ cells in tuberculoid and lepromatous lesions (mean  $\pm$  S.D.).

Cell type	Tuberculoid (N = 8)	Lepromatous (N = 8)
OKT6+/100 basal nuclei	28.2 $\pm$ 3.5	15.3 $\pm$ 3.3
% IL-2-producing cells <sup>a</sup>	0.032 $\pm$ 0.037	0.0019 $\pm$ 0.023
% IL-2-receptor+ cells <sup>b</sup>	11.7 $\pm$ 7.8	1.9 $\pm$ 1.5

<sup>a</sup> % IL-2-producing cells = number of cells showing intense cytoplasmic staining with the DMS-1 monoclonal antibody/mm<sup>2</sup> of granuloma divided by the number of T cells (Leu1+ cells)/mm<sup>2</sup> of granuloma  $\times$  100.

<sup>b</sup> % IL-2-receptor+ cells = number of cells reacting with the anti-Tac monoclonal antibody/mm<sup>2</sup> of granuloma divided by the number of Leu1+ cells/mm<sup>2</sup> of granuloma  $\times$  100.

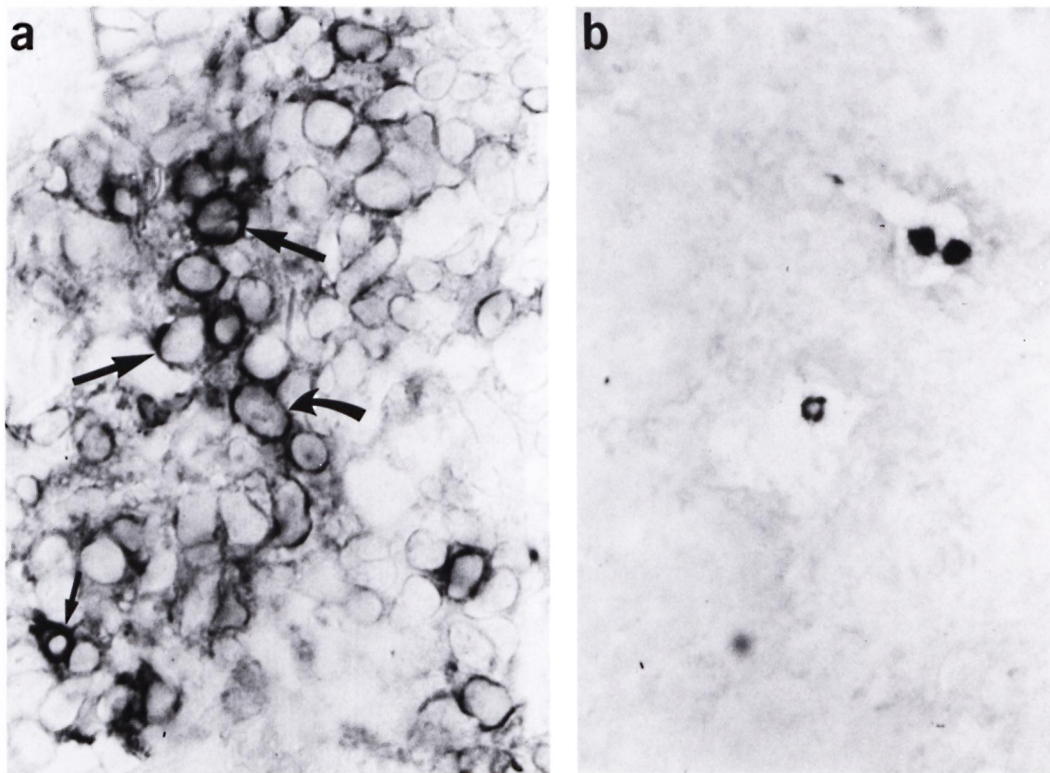


FIG. 4. Tuberculoid leprosy granuloma. **a)** Anti-Tac<sup>+</sup> cells stained blue with alkaline phosphatase reaction product. Leu3<sup>+</sup> lymphocytes stained with a dark brown rim of horseradish peroxidase reaction product. Anti-Tac<sup>+</sup> cells have variable staining intensity and morphology, ranging from small, darkly staining round cells (small arrow = Leu3<sup>+</sup>, Tac<sup>+</sup> cell) to larger, blast-like cells (Leu3<sup>-</sup>, Tac<sup>+</sup> cell = curved arrow; Leu3<sup>+</sup>, Tac<sup>+</sup> cells = large straight arrows and unmarked). Original color photomicrographs clearly demonstrate these differences (horseradish peroxidase and alkaline phosphatase  $\times 576$ ). **b)** Cytoplasmic staining of unbound IL-2 in cells of a tuberculoid leprosy granuloma using the DMS-1 monoclonal antibody and the avidin-biotin horseradish peroxidase complex technique ( $\times 576$ ).

absolute numbers of T cells, lymphocyte subset ratios (mean OKT4-Leu3/OKT8 = 1.4), or percentage of cells expressing MCH Class II antigens in the peripheral blood in the different clinical groups. These results differ from those reported previously by our laboratory<sup>(15)</sup> and others<sup>(21)</sup>, and may be due to differences in methods, sample size limitations, or subject variability.

#### DISCUSSION

The results presented here show that macrophage activation and host resistance to *M. leprae* is most significantly correlated with the absolute number of cells bearing the OKT4-Leu3 phenotype in lesions. There is a strong correlation between the number of OKT6<sup>+</sup> Langerhans' cells and the percent-

age of lymphocytes bearing the IL-2 receptor in leprosy lesions. The majority of interleukin-2 receptor-bearing cells in untreated naturally occurring lesions express the Leu3 phenotype. The correlation between OKT6<sup>+</sup> and IL-2-producing cells is positive, but it is less strong than the correlation between OKT6<sup>+</sup> cells and IL-2 receptor-bearing cells. As we stated earlier, we found that the change in the absolute number of OKT4<sup>+</sup>-Leu3<sup>+</sup> (T helper cells) across the clinical spectrum of leprosy accounts for the change in the OKT4-Leu3/OKT8-Leu2 ratio observed by others<sup>(14, 16, 27)</sup>.

The absolute number of OKT8<sup>+</sup> cells in the lesions is not associated with host resistance in our study. The inability of the immune system to activate cells of the

monocyte-macrophage series to destroy phagocytosed *M. leprae* is the central defect in multibacillary forms of leprosy. We have hypothesized<sup>(8)</sup> that this is caused by a failure of IL-2 production which results in poor expansion of T-cell clones, and this results in a lack of T cell "help" with poor macrophage function and poor destruction and removal of the bacilli. Since at least some lepromatous patients have lymphocytes which are capable of proliferation *in vitro* in response to *M. leprae* if IL-2 is provided, and since we have shown that IL-2 production is decreased *in situ*, the hypothesis of failure of clonal proliferation is supported.

We conclude, therefore, that the poor destruction of *M. leprae* in lepromatous disease results from decreased production of IL-2, which causes failure of T-cell clonal proliferation and probably a lack of generation of macrophage activating factors. Whether the decreased production of IL-2 occurs because of active suppression or some other mechanism is not clear from these studies.

Recently, Modlin, *et al.* published findings<sup>(13)</sup> which support our main hypothesis. They demonstrated IL-2-producing cells in naturally occurring lesions of tuberculoid leprosy, but they found significantly fewer of these cells in lepromatous lesions. Like us, the authors found approximately equal absolute numbers of OKT8-Leu2+ cells at both ends of the spectrum and a significantly greater number of OKT4-Leu3+ cells in tuberculoid lesions. However, they focused on the ratios of Leu2/OKT8 to Leu3/OKT4 cells in the lesions and speculated that "The relative excess of Leu2a/OKT8+ cells in the lepromatous granuloma may contribute to both the disorganization of and relatively decreased IL-2 production by this granuloma." We, for the first time, are drawing attention to the absolute lack of Leu3/OKT4 cells in the lepromatous granulomas. While some OKT8+ cells may indeed act to suppress the immune reaction in lepromatous leprosy, we suggest that many of the Leu2/OKT8+ cells may be part of the normal immune response and that the decreased Leu3/OKT4 to Leu2/OKT8 ratios in lepromatous lesions may be the result of poor production of IL-2 rather than the cause of it.

The findings of Modlin, *et al.*<sup>(13)</sup> using

anti-Tac are similar to ours in lepromatous leprosy; both studies found approximately the same percentage of small round Tac+ cells. A difference, however, is that we demonstrated a significantly higher percentage of Tac+ cells in tuberculoid lesions. The reasons for this discrepancy are not clear and may be due to methodological differences between the two studies. Their results are reported as the percentage of the total cells in the granuloma, whereas we report the number of Tac+ cells as a percentage of Leu1+ lymphocytes. More importantly perhaps, we used a higher concentration of anti-Tac antibody which gave a lower signal to noise ratio but allowed us to detect cells expressing the Tac antigen at a lower density. Since populations of stimulated and dividing cells may express the IL-2 receptor to a variable degree and since some of the Tac+ cells identified in tuberculoid lesions were large and resembled blasts, we believe that the population of cells we stained represent proliferating cells and is immunologically important.

These same authors pointed out the close apposition of OKT6+ cells with IL-2-producing cells. They, and others<sup>(2, 14, 22, 27)</sup>, have previously noted differences in the numbers and microanatomic distribution of Langerhans' cells in different types of leprosy. We have also occasionally seen this spatial association of OKT6+ cells and IL-2-producing cells, and we analyzed our data to test the association of these two cell types. Although there is an association between four cell types (OKT6+, Leu3-OKT4+, IL-2 receptor+, and IL-2-producing cells), the strongest correlation is between OKT6+ cells and IL-2 receptor+ cells. Since antigen presentation is believed to be required for IL-2 receptor expression in all but extremely restricted *in vitro* situations, and since OKT6+ cells are associated with antigen presentation, we suggest that one of the main functions of OKT6+ cells in leprosy lesions is to promote the expansion of T-cell clones by inducing expression of the IL-2 receptor. Furthermore, we found this antigen-presenting system apparently acting at a higher level in naturally occurring tuberculoid lesions than in lepromatous lesions. We must stress, however, that we have merely demonstrated an association, and our findings in no way preclude the role of Lan-

gerhans' cells in the generation of IL-2. Proof of causal relationships will probably require *in vitro* studies.

Several points about our data are worth mentioning. The standard deviations of the mean percentage of IL-2-producing cells are large in comparison to the actual mean values. This does not appear to be simply a consequence of the relatively small number of lesions examined, but to represent real variability in tuberculoid lesions. The percentage of IL-2-producing cells in lepromatous patients was always below the mean value seen in tuberculoid patients. The values for individual tuberculoid patients showed greater absolute variation. Some values were quite high, and others were within the range of values for lepromatous patients. Since all patients examined showed at least one IL-2-producing cell in their granuloma, we do not believe that technical factors explain this variation. One possible explanation is that the tuberculoid patients may represent a heterogeneous population. Since the lesions of borderline and tuberculoid patients may be evolving, this heterogeneity may simply represent the fact that the patients were biopsied at different points in their disease, perhaps some in a phase when the proliferative response was not active. Of course, there are other possible explanations for this heterogeneity.

### SUMMARY

To investigate the immune defect in lepromatous leprosy we studied immune cell phenotypes, lymphocyte activation states, and interleukin-2 (IL-2) production in naturally occurring leprosy skin lesions. Mouse hybridoma monoclonal antibodies reacting with the IL-2 receptor (anti-Tac), unbound IL-2 (DMS-1), antigen-presenting Langerhans' cells (OKT6) and the OKT4-Leu3 and OKT8 T-lymphocyte subpopulations were used with indirect horseradish peroxidase and alkaline phosphatase techniques on frozen biopsy sections. The percentage of Tac+ lymphocytes and the number of OKT6+ cells in the epidermis and dermal granuloma were significantly correlated in naturally occurring lesions (correlation coefficient 0.79) and were higher in tuberculoid than in lepromatous lesions. Leu3 antigen was expressed by 70–90% of Tac+ cells in

tuberculoid lesions. Although the percentage of cells producing IL-2 was low in lesions of both lepromatous and tuberculoid patients, it was about 15 times greater in tuberculoid than in lepromatous lesions ( $0.032 \pm 0.037$  tuberculoid vs  $0.0019 \pm 0.023$  lepromatous). There was an association between the number of OKT6+ cells and the percentage of IL-2-producing cells, but the association was weaker than that of OKT6+ cells and the percentage of IL-2 receptor-bearing cells ( $r = 0.2$ ), implying that IL-2 production is not an intervening variable in the latter association. The absolute number of OKT4-Leu3+ lymphocytes was significantly different in different clinical leprosy groups and was positively correlated with host resistance (mean OKT4-Leu3+ cells/mm<sup>2</sup> in 6  $\mu$ m sections;  $1412 \pm 288$  tuberculoid,  $400 \pm 93$  borderline lepromatous,  $200 \pm 100$  polar lepromatous;  $r = 0.95$ ). Absolute numbers of OKT8+ cells/mm<sup>2</sup> in lesions were not significantly different.

We conclude that there is a relative paucity of OKT4-Leu3+ cells as well as IL-2-producing cells at the local level in lepromatous leprosy lesions. Possible functional relationships between these findings and the failure of macrophage activation and destruction of *Mycobacterium leprae* in lepromatous leprosy are discussed.

### RESUMEN

Con el fin de estudiar el defecto inmunológico en la lepra lepromatosa, se examinaron los fenotipos de las células inmunes, los estados de activación de los linfocitos, y la producción de interleucina 2 (IL-2), en las lesiones dérmicas de pacientes lepromatosos. Se usaron anticuerpos monoclonales murinos contra el receptor para IL-2 (anti-Tac), contra IL-2 libre (DMS-1), contra células de Langerhans presentadoras de antígeno (OKT6), y contra las subpoblaciones de linfocitos T, OKT4-Leu3 y OKT8. El porcentaje de linfocitos Tac+ y el número de células OKT6+ en la epidermis y en los granulomas dérmicos mostraron una correlación significativa (coeficiente de correlación 0.79) y fueron mayores en las lesiones tuberculoides que en las lepromatosas. El antígeno Leu3 fue expresado por el 70–90% de las células Tac+ en las lesiones tuberculoides. Aunque el porcentaje de células productoras de IL-2 fue bajo tanto en las lesiones lepromatosas como en las tuberculoides, fue casi 15 veces mayor en las lesiones tuberculoides que en las lepromatosas ( $0.032 \pm 0.037$  y  $0.0019 \pm 0.023$ , respectivamente). Hubo asociación entre el número de células OKT6+ y el por-



centaje de células productoras de IL-2 pero la asociación fue menor que aquella entre las células OKT6+ y las células Tac+ ( $r = 0.2$ ), implicando que la producción de IL-2 no es una variable que participe en la última asociación. El número absoluto de linfocitos OKT4-Leu3+ fue significativamente diferente en los diferentes grupos clínicos de lepra y correlacionó positivamente con la resistencia del huésped (promedio de células OKT4-Leu3+ por mm<sup>2</sup> en secciones de 6  $\mu$  en lesiones tuberculoïdes:  $1412 \pm 288$ , lepromatosas intermedias:  $400 \pm 93$ , lepromatosas polares:  $200 \pm 100$ ,  $r = 0.95$ ). Los números absolutos de células OKT8+ por mm<sup>2</sup> en las lesiones no difirieron significativamente.

Concluimos que hay una relativa carencia de células OKT4-Leu3+ y de células productoras de IL-2 en las lesiones de la lepra lepromatosa. Se discuten las posibles relaciones funcionales entre estos hallazgos y la falla de los macrófagos para activarse y destruir al *Mycobacterium leprae* en la lepra lepromatosa.

### RÉSUMÉ

En vue d'explorer les déficiences immunologiques dans la lèpre lépromateuse, on a étudié les phénotypes des cellules immunitaires, les états d'activation des lymphocytes, et la production d'interleukine 2 (IL-2), dans des lésions cutanées de lèpre apparues naturellement. On a utilisé des anticorps monoclonaux provenant d'hybridomes de la souris, réagissant avec le récepteur pour IL-2 (anti-Tac), avec l'IL-2 libre (DMS-1), avec les cellules de Langerhans présentant les antigènes (OKT6), et avec les sous-populations de lymphocytes T OKT4-Leu3 et OKT8; ces études ont été menées sur des coupes de biopsies congelées, par des techniques indirectes utilisant la peroxydase de raifort et la phosphatase alcaline. La promotion de lymphocytes Tac+ et le nombre de cellules OKT6+ dans l'épiderme et dans les granulomes du derme présentaient une corrélation significative dans les lésions apparues naturellement (coefficient de corrélation 0,79); les chiffres étaient plus élevés pour les lésions de lèpre tuberculoïde que pour les lésions de lèpre lépromateuse. L'antigène Leu3 était exprimé au niveau de 70–90% des cellules Tac+ dans les lésions des malades tuberculoïdes. Quoique le pourcentage de cellules produisant de l'interleukine 2 soit faible dans les lésions, tant les lésions provenant de malades lépromateux que des malades tuberculoïdes, il est encore 15 fois plus élevé chez les tuberculoïdes que chez les lépromateux ( $0,032 \pm 0,037$  chez les tuberculoïdes et  $0,0019 \pm 0,023$  lépromateux). On a relevé une association entre le nombre de cellules OKT6+ et le pourcentage de cellules produisant de l'interleukine 2; cette association était cependant moins marquée que celle notée entre les cellules OKT6+ et le pourcentage de cellules porteuses du récepteur pour l'interleukine 2 ( $r = 0,2$ ). Ceci démontre que la production d'interleukine 2 n'est pas une variable déterminante dans cette association. Le nombre absolu de lymphocytes OKT4-Leu3+ était significativement différent selon les divers types cliniques

de lèpre; ce nombre présentait une corrélation positive avec la résistance de l'individu (les moyennes du nombre de cellules OKT4-Leu3+/mm<sup>2</sup> dans des sections de 6  $\mu$  étaient les suivantes:  $1412 \pm 288$  chez les tuberculoïdes,  $400 \pm 93$  chez les lépromateux dimorphes,  $200 \pm 100$  chez les lépromateux polaires; avec un coefficient de corrélation ( $r = 0,095$ ). Les nombres absolus de cellules OKT8+/mm<sup>2</sup> dans les lésions n'étaient pas significativement différents.

On en conclut que dans les lésions de lèpre lépromateuse les cellules OKT4-Leu3+, de même que celles produisant de l'interleukine 2, sont relativement rares au niveau local. On discute des relations fonctionnelles qui pourraient exister entre ces observations d'une part, et d'autre part l'absence d'activation des macrophages et de destruction de *Mycobacterium leprae* dans la lèpre lépromateuse.

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