

Natural Emergence of Antigen-Reactive T Cells in Lepromatous Leprosy Patients during Erythema Nodosum Leprosum

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Fifteen lepromatous leprosy (LL) patients undergoing erythema nodosum leprosum (ENL) reactions were compared with 13 stable, uncomplicated, anergic individuals of the same leprosy background. ENL patients showed significant antigen-induced leukocyte migration inhibition (migration index = 0.058 ± 0.01), paralleling the values obtained with a responder tuberculoid leprosy population (migration index = 0.04 ± 0.004). Both phytohemagglutinin-induced general T-cell proliferation and, more significantly, antigen-induced lymphoproliferation were enhanced during the acute phase of the reaction. Suppressor cell activity, monitored by a costimulant assay, showed enhanced antigen-stimulated suppression of mitogen responses. Interestingly, the improvement in in vitro T-cell responses was not reflected in dermal reactivity, since 48-h delayed-type hypersensitivity responses after intradermal injection of soluble *Mycobacterium leprae* antigens continued to be poor. After subsidence of reactional lesions, leukocyte migration inhibition, lymphoproliferation, and suppressor cell activity were reduced to the unresponsive state seen in stable LL patients. Significantly, perturbations of T-cell reactivity are detectable in ENL reactions, indicating the natural but transient emergence of antigen-induced T cells in LL.

Lepromatous leprosy (LL), the disseminated form of *Mycobacterium leprae* infection, exhibits selective, antigen-specific T-cell unresponsiveness. LL patients have poor delayed-hypersensitivity reactions and reduced lymphoproliferation in response to T-cell mitogens (3, 12) and *M. leprae* antigens (7, 18). The nonspecific unresponsiveness noted in some LL patients is reversed after chemotherapy, though specific antigen-related anergy is persistent and long lasting (12, 19). Enhanced B-cell functions have been reported in LL (18). Immunoregulatory abnormalities due to enhanced (11) or decreased (20, 22, 23, 32, 34) suppressor T-cell activity and macrophage- (30) or monocyte-derived (31) factors have been implicated in pathogenic mechanisms underlying the defect in LL.

LL patients suffer from acute, episodic, exacerbated reactional states called erythema nodosum leprosum (ENL), which are characterized by crops of tender, erythematous, subcutaneous nodules and systemic manifestations of pyrexia, malaise, neuritis, arthralgia, and proteinuria. The mechanisms underlying ENL have been attributed to immune complex-mediated injury as evidenced by histopathology (37), presence of immunoglobulin and complement in lesions (36), and circulating immune complexes (1, 5, 14) and complement-degradative products (1, 6).

In recent years, sporadic evidence of altered T-cell reactivity in patients with ENL has been observed by us (22) as well as others (27). The present study was undertaken in LL patients to systematically evaluate the status of T-cell-mediated functions during and after ENL reactions. Definitive evidence for transient improvement in T-cell functions was obtained in many LL patients during the acute phase of ENL. Significantly, antigen-induced (i) leukocyte migration inhibition, (ii) lymphoproliferation, and (iii) suppressor T-cell activity were enhanced, indicating the presence or emergence of antigen-reactive T cells in LL patients. After subsidence of ENL lesions, most patients showed a return to hyporeactivity or anergy associated universally with LL.

Interestingly, the transient improvement in in vitro T-cell functions was not associated with the appearance of skin reactivity to concomitant intradermal injection of soluble *M. leprae* antigens.

MATERIALS AND METHODS

Patients. Twenty-eight patients classified by the Ridley and Jopling (29) scale were included in the study. Thirteen patients had uncomplicated LL, whereas 15 presented with erythematous, subcutaneous, tender crops of nodules on an LL background and were taken as ENL patients. The clinical details are given in Table 1.

Immunological studies were performed (i) during active ENL, before initiation of antireaction therapy, as well as (ii) 1 week to 4 months after stopping treatment, when clinical signs and symptoms of ENL had subsided. All patients were subsequently treated with aspirin, thalidomide (300 mg daily), and clofazimine (300 mg daily), with or without prednisolone (20 to 30 mg daily). All drugs were gradually reduced, and patients were maintained on clofazimine (100 mg on alternate days) along with dapsone (100 mg daily) and rifampin (1,200 mg monthly).

Stimulants. (i) Autoclaved, armadillo-derived *M. leprae* (lepromin A), kindly supplied by R. J. W. Rees, NIMR, London, was used in the leukocyte migration inhibition test. (ii) *M. leprae* extracted from skin biopsies from bacilliferous LL patients as described earlier (25) were used for the lymphoproliferative and suppressor cell assay. In brief, the tissue was homogenized in glass homogenizers in RPMI 1640 after prior removal of the epidermis. Extracted bacilli were screened for the absence of other contaminating bacteria by culturing on (i) nutrient agar for 72 h and (ii) Lowenstein-Jensen medium for 8 weeks. The bacilli were counted by the standard method described earlier (8). Three concentrations of antigen were used for each patient for lymphoproliferative assays; 1.25×10^4 , 1.25×10^5 , and 1.25×10^6 *M. leprae* were added for 100 μ l of 10^5 peripheral blood monocytes (PBM) per well. The data are expressed for the optimal response at a given antigen concentration. In general, 1.25×10^5 bacilli

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TABLE 1. Clinical details of 15 ENL patients

Clinical signs and symptoms	% Patients affected
Subcutaneous nodules	
Erythema	100
Tenderness	86
Nerves (1-4)	
Thickening	66
Tenderness	40
Constitutional symptoms	
Fever	86
Joint pains	73
Edema	53
Hypoanesthesia	40
Eye involvement	13

per well was optimal for lymphocyte responses of responder tuberculoid patients. (iii) Sonicated *M. leprae* (10^8 bacilli per ml) were prepared in an ultrasonic power unit (M. S. E. Scientific Instruments) for 60 min. Dilutions corresponding to the bacterial concentrations mentioned above were prepared in RPMI 1640 medium and filter sterilized (0.45- μ m [pore size] membrane filter; Millipore Corp., Bedford, Mass.) before use. (iv) Phytohemagglutinin-P (PHA-P; Sigma Chemical Co., St. Louis, Mo.) was used at optimal (200 μ g/ml) and suboptimal (20 μ g/ml) concentrations (as tested on control subjects) for both lymphoproliferative and suppressor cell assays.

Leukocyte migration inhibition assay. The methodology used was a modification of the techniques described earlier (10). Leukocytes obtained from heparinized (10 IU of preservative-free heparin; Microlabs, Bombay, India) venous blood were washed twice with Hanks balanced salt solution (Microlabs) and suspended at 4×10^7 to 5×10^7 cells per ml in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 100 IU of penicillin per ml, and 100 μ g of streptomycin per ml. One portion of the cell suspension was incubated with medium only (RPMI 1640), and the other was incubated with 10^8 *M. leprae*. Both portions were incubated at 37°C for 1 h with humidified 5% CO₂ in air. Subsequently, 20- μ l capillaries (Arthur H. Thomas Co., Philadelphia, Pa.) were charged with test and control cell suspensions, centrifuged at low speed for 2 min, and cut just below the cell-supernatant interphase. Cells were then allowed to migrate for 16 h at 37°C in migration chambers (Laxbro; Pune, India) filled with RPMI 1640 and 10% fetal calf serum (Microlabs) in an atmosphere of 5% CO₂ in air. Four replicates of control and antigen-treated leukocytes were set up. The area of migration of leukocytes was measured by projection on a screen. The migration index (MI) was calculated as: MI = (area of migration with antigen)/(area of migration without antigen).

Separation of PBMC. PBMC were isolated from sterile, heparinized venous blood by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) density gradient centrifugation (2). After the cells were washed twice with Hanks balanced salt solution, they were suspended at a concentration of 10^6 cells per ml in RPMI 1640 containing 10% autologous plasma.

Lymphoproliferation assay. Quadruplicates of 10^5 PBMC in 100 μ l of medium and 25 μ l of (i) medium, (ii) suboptimal

PHA-P, (iii) optimal PHA-P, (iv) integral antigen at 1.25×10^5 , (v) integral antigen at 1.25×10^6 , (vi) integral antigen at 1.25×10^7 , and (vii, viii, and ix) sonicated antigens corresponding to iv, v, and vi were set up in round-bottomed microculture plates (Nunc, Roskilde, Denmark). The cells were incubated at 37°C in 5% CO₂ and air. Mitogen and antigen cultures were terminated after 72 and 144 h, respectively. The cells were harvested onto glass fiber discs by a semiautomatic cell harvester (Ilacon, Tonbridge, U.K.) 18 to 20 h after the addition of 0.5 μ Ci of [³H]thymidine (specific activity, 1.80 Ci/mmol; Bhabha Atomic Research Centre, Trombay, India). Radioactivity was counted in a Rackbeta II 1215 scintillation counter (LKB Instruments, Inc., Rockville, Md.). The counts per minute (cpm) \pm the standard error of replicate cultures were calculated, and the degree of proliferation (Δ cpm) was derived as: Δ cpm = cpm in stimulated cultures - cpm in unstimulated cultures.

Suppressor cell assay. The costimulant assay described earlier (22) from our laboratory was used with the proviso that PHA-P was used as a mitogen instead of concanavalin A (Pharmacia). In brief, quadruplicate cultures were set up. One-hundred-microliter volumes of 10^5 PBMC per well were cultured with (i) 25 μ l of RPMI 1640, (ii) 25 μ l of integral or sonicated *M. leprae* antigen (5×10^6 bacilli per ml), (iii) 25 μ l of antigen followed by 25 μ l of PHA-P (20 μ g/ml), (iv) 200 μ l/ml 24 h later, or (v) 25 μ l of PHA-P at the same time as in iii and iv. Cultures were harvested at 72 h after mitogen addition, after 18 to 20 h or prior pulsing with 0.5 μ Ci of [³H]thymidine as described above. Percent suppression was calculated as follows: Percent suppression [(cpm with mitogen - cpm with mitogen-antigen)/(cpm with mitogen + cpm with antigen)] \times 100.

Characterization of PBMC. T cells. Indirect immunofluorescence with murine monoclonal antibodies (OKT series, orthoclone; Ortho Diagnostics, Inc., Raritan, N.J.) and fluorescein isothiocyanate-conjugated F(ab')₂ anti-mouse fragments (New England Nuclear Corp., Boston, Mass.) were used for enumerating T cells and their subsets. Five microliters of OKT3 (pan T cell), OKT4 (helper-inducer subset), and OKT8 (suppressor-cytotoxic subset) was added to 10^6 PBMC in 100 μ l of RPMI 1640 containing no plasma. The cells were incubated for 30 min at 4°C, washed twice, suspended in a 100- μ l volume, and exposed to 5 μ l of the second antibody for 60 min at 4°C. Subsequently, the cells were washed twice and counted by viewing under a Zeiss universal microscope (Carl Zeiss, Oberkochen, Federal Republic of Germany) with epi-illumination with an HB0 50 mercury lamp, excitation filter BP 450-490, and barrier filter BP 520-560. A minimum of 200 cells were counted for each sample.

B cells. The presence of surface immunoglobulin was used for B-cell enumeration. Briefly, 10^5 cells were exposed to 50 μ l of 1:40 fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulins (immunoglobulin G [IgG] + IgM + IgA; Cappel Laboratories, Cochranville, Pa.) for 60 min at 4°C, washed, and enumerated as described above.

Skin test. Soluble, armadillo-derived *M. leprae* antigen (100 μ l) (leprosin A; 10 μ g/ml) was injected intradermally on the volar aspect of the forearm. Induration was measured 48 h later, and diameters of <10 mm were regarded as negative.

Statistical analysis. The data were analyzed by the Mann-Whitney *U* test (33).

RESULTS

The clinical features of 15 LL patients undergoing ENL reactions are given in Table 1. Of these patients, 7 were

TABLE 2. In vitro responses in tuberculoid and LL patients

Diagnosis	MI	Mean \pm SEM (no. of patients)				% Suppression with:	
		Δ cpm with:				IB ^c	SB ^d
		PHA-P(s) ^a	PHA-P(o) ^b	IB ^c	SB ^d		
Tuberculoid leprosy	0.04 \pm 0.004 (6)	7,657 \pm 1,146 (8)	31,385 \pm 3,837 (8)	3,317 \pm 489 (9)	3,767 \pm 458 (7)	57.7 \pm 6.7 (8)	48.3 \pm 4.9 (7)
LL	0.86 \pm 0.10 (8)	662 \pm 187 (10)	14,544 \pm 2,833 (9)	51 \pm 96 (9)	85 \pm 89 (8)	10.8 \pm 7.5 (9)	10.78 \pm 3.6 (9)

^a Suboptimal PHA-P concentration.^b Optimal PHA-P concentration.^c IB, Integral bacilli.^d SB, Sonicated bacilli.

available for follow-up studies 1 week to 4 months after subsidence of clinical signs of ENL and completion of specific antireaction therapy. Treatment with combination antileprosy drugs was continued throughout. For comparison, 13 LL patients with the stable form of the disease and without a history of earlier reactions were included in the study. Tuberculoid leprosy patients formed the baseline control group used to evaluate the level of responsiveness to

M. leprae antigens in leprosy as a whole. Integral and membrane-filtered sonicated bacillary antigens were used to evaluate responses to surface and cytoplasmic antigens, respectively. Three concentrations of the antigens corresponding to suboptimal, optimal, and supraoptimal bacillary numbers were used for both antigens to cover any dose-related differences in the lymphoproliferative responses. Only optimal responses are included in the results.

Nonreactional, stable leprosy. Whereas tuberculoid leprosy patients showed optimal responses to PHA-P and *M. leprae* antigens (Table 2), all LL patients showed a lack of antigen-induced leukocyte migration inhibition (Fig. 1a) and lymphoproliferative in vitro responses to soluble and integral *M. leprae* antigens (Fig. 1c). Moreover, the majority of LL patients showed poor mitogen responses (Fig. 1b). In conformity with our earlier observations with concanavalin A as mitogen (22), stable LL patients showed no or poor inhibition of mitogen responsiveness in the presence of both types of *M. leprae* antigen (Fig. 1d).

Active ENL. Leukocyte migration inhibition test. Nine patients tested during active ENL and before institution of specific antireaction therapy gave evidence of strong antigen-induced leukocyte migration inhibition in response to heat-killed integral *M. leprae* (Fig. 1a). The mean MI for the ENL group (MI = 0.058 \pm 0.01) was significantly different ($P < 0.005$) from that of control LL patients who showed the expected unresponsiveness (mean MI = 0.86 \pm 0.10). Interestingly, the level of migration inhibition in the ENL group matched the levels attained in responder tuberculoid leprosy individuals (Table 2). Three patients tested within a week of initiation of antireaction therapy showed abrogation of migration inhibition (mean MI = 0.39 \pm 0.10).

Lymphoproliferative responses. We evaluated mitogen-induced lymphoproliferation at both suboptimal (20 μ g/ml) and optimal (200 μ g/ml) concentrations of PHA-P with a view to cover dose-related alterations, if any, in T-cell responsiveness during acute ENL. Some ENL patients showed enhanced mitogen responsiveness compared with the nonreactional group at both concentrations of PHA-P (Fig. 1b). The mean Δ cpm \pm the standard error for the ENL

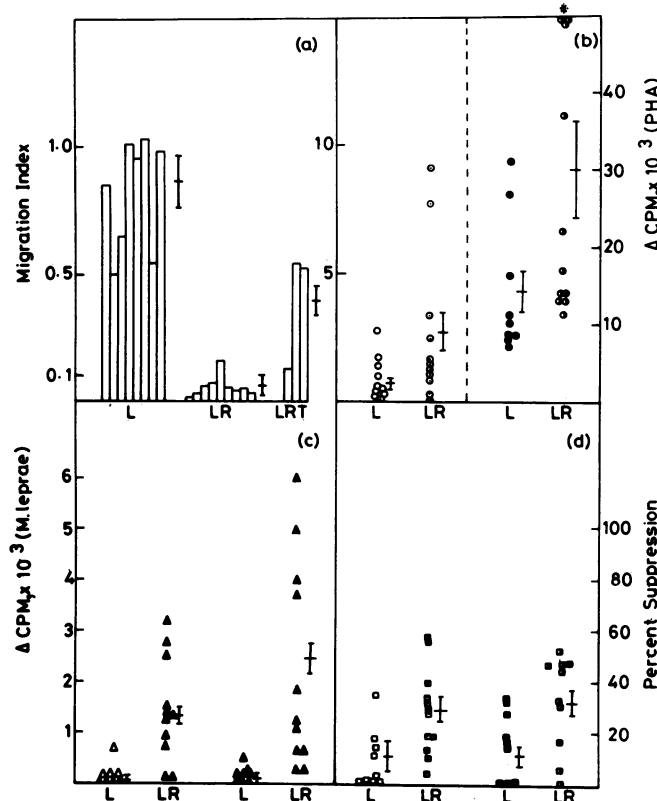


FIG. 1. Immunological responses in LL (L) and ENL (LR) patients. (a) Leukocyte migration inhibition factor production in the presence of *M. leprae* antigens. MI, Ratio of area of migration of leukocytes in the presence and absence of antigen. (b) Lymphoproliferative response of PBMC in the presence of suboptimal (○, ○) and optimal (●, ●) concentrations of PHA-P. Δ cpm, cpm in stimulated cultures minus cpm in unstimulated cultures. (c) Lymphoproliferative response of PBMC in the presence of integral (Δ, Δ) and sonicated (▲, ▲) *M. leprae* antigens. (d) Suppressor cell

activity generated by integral (□, □) and sonicated (■, ■) *M. leprae* antigens as assessed by their effect on lymphoproliferation in response to a suboptimal concentration of PHA-P. Percent suppression was calculated as: percent suppression = [(cpm in the presence of mitogen - cpm in the presence of antigen-mitogen)/(cpm in the presence of mitogen + cpm in the presence of antigen)] \times 100. Values obtained with individual patients are depicted by vertical lines showing the mean \pm standard error of the mean. *, Values above 50,000 cpm.

TABLE 3. Characterization of PBMC from LL and ENL patients

Leprosy type (no. of patients)	Range (mean \pm SE) of % of cells characterized as:			
	OKT3 ⁺	Sig ⁺ ^a	OKT4 ⁺	OKT8 ⁺
LL (10)	48-80 (59.7 \pm 1.7)	18-26 (21.5 \pm 1.0)	25-51 (33.7 \pm 2.5)	12-38 (22.4 \pm 2.86)
ENL (9)	58-80 (67.67 \pm 1.8)	21-27 (22.8 \pm 0.09)	16-41 (30.6 \pm 0.96)	31-52 (38.11 \pm 2.46)

^a Sig⁺ cells are B cells.

group were 2,669 \pm 809 and 30,478 \pm 6,707, respectively, for the lower and higher concentrations of PHA-P.

Of greater significance was the detection of antigen-induced in vitro lymphoproliferation to *M. leprae* antigens of 9 of 11 patients undergoing ENL reactions (Fig. 1c). Responsiveness to both integral and soluble antigens in the ENL group was significantly higher ($P < 0.01$) compared with the uncomplicated LL group. However, the degree of proliferation observed in ENL patients was relatively lower than that seen in the tuberculoid responder group (Table 2). The mean Δ cpm with integral and soluble antigens in the ENL group were 1,331 \pm 286 and 2,495 \pm 557, respectively.

Suppressor cell activity. Antigen-induced suppressor cell activity was assessed in a costimulant assay as described earlier (22), whereby the effect of integral and sonicated antigens on mitogen responses of the patients were graded. Fig. 1d gives individual data for both antigens for both groups of patients. The mean percent suppression in the stable LL group was 10.8 \pm 7.1 and 10.78 \pm 3.59% with integral and soluble antigens, respectively. ENL patients showed significant enhancement of suppression, the mean percent suppression being 28.9 \pm 4.7 and 32.4 \pm 5.4% for integral ($P < 0.01$) and sonicated ($P < 0.05$) antigens, respectively. This increase in suppression noted in ENL was only observed when suboptimal PHA-P was used. The degree of suppression induced by the two antigen preparations showed no differences between the stable LL and ENL groups when the optimal PHA-P concentration was used in the assay.

Lymphocyte subsets. T-cell subsets in the PBMC of both groups of patients were characterized by indirect immunofluorescence with monoclonal antibodies OKT3, OKT4, and OKT8. Nine ENL patients showed an increase in OKT8⁺ cells with a significantly ($P < 0.05$) lower OKT4/OKT8 ratio (0.81 \pm 0.1) compared with that observed in patients with uncomplicated LL (1.68 \pm 0.25) (Table 3). B-cell percentages in the PBMC of ENL patients were similar to those of uncomplicated LL individuals (Table 3).

Delayed-hypersensitivity reaction. All patients with ENL showed absence of erythema and induration 48 h after intradermal injection of soluble armadillo-derived *M. leprae* antigens (10 μ g/ml). This lack of responsiveness paralleled the anergy observed in nonreactive LL patients (Table 4).

Post-ENL status. Of the ENL patients, 7 were tested after subsidence of lesions and termination of reaction-specific

TABLE 4. Comparison of delayed-type hypersensitivity responses to leprosin A and leukocyte migration inhibition test performed with lepromin A in ENL patients^a

Leprosy type	Skin induration diam (mm)	MI
LL	0-0 (0 \pm 0) (7)	0.39-1.34 (0.86 \pm 0.10) (8)
ENL	0-0 (0 \pm 0) (9)	0.01-0.16 (0.06 \pm 0.01) (9)

^a First set of values in parentheses is the mean \pm standard error; second set of values in parentheses is the number of patients.

therapy. All patients showed a reversal of the T-cell reactivity observed during active ENL (Fig. 2). The levels of MI, (Fig. 2a), lymphoproliferative responses (Fig. 2b and c), and suppressor activity (Fig. 2d) in post-ENL patients were similar to those observed in the control group of patients with uncomplicated LL.

DISCUSSION

The present study provides evidence for the natural emergence of transient T-cell reactivity in hitherto anergic LL patients during the reactional phase of ENL (16). Significantly, both lymphoproliferative and lymphokine-related in vitro T-cell responses to *M. leprae* antigens were detectable in many ENL patients. The degree of leukocyte migration inhibition in the acute phase of the reaction was similar to that obtained with cells from responder tuberculoid leprosy patients. Of the 11 ENL individuals, 9 showed significant lymphoproliferative responses in the presence of both solu-

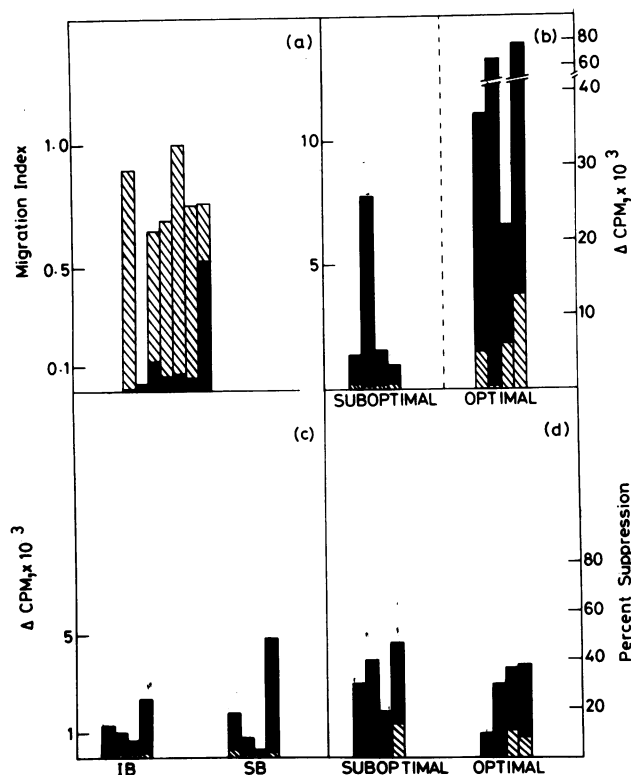


FIG. 2. Immunological studies during the postreactional phase in ENL patients. Symbols: ■, During reaction; ▨, postreaction. (a) MI. (b) Δ cpm with suboptimal and optimal concentrations of PHA-P. (c) Δ cpm with integral (IB) and sonicated (SB) bacilli. (d) Percent suppression at suboptimal and optimal concentrations of PHA-P. Values obtained with individual patients are depicted.

ble and integral *M. leprae* antigens. However, the level of proliferation was of a moderate degree and did not reach the levels usually observed with tuberculoid leprosy individuals. Our results also confirm earlier reports of enhanced mitogenic responses in LL patients undergoing ENL reactions (15, 35). ENL lesions are known to frequently show fragmented bacilli (28). It is possible that the transient increase in T-cell reactivity may play a role in bacterial killing.

Suppressor T-cell activity was evaluated in leprosy patients in a costimulant assay (22), whereby the effect of antigens on the mitogenic responses of PBMC was measured. We (20, 22, 23), as well as others (4, 32, 34), had reported that LL patients showed low or absent *M. leprae*-induced suppressor cell activity on concanavalin A responses during the stable form of the disease compared with healthy responder individuals and tuberculoid patients. However, one report showed contrary results of increased T8⁺ suppressor T-cell function in LL (11). With a view to investigating further the suppressor mechanisms in this disease, we used PHA as the T-cell mitogen and soluble and integral human-derived *M. leprae* as antigens in the *in vitro* assay. The present results confirm our earlier findings of a lack of suppressor T-cell activity in the stable form of the disease. Interestingly, suppressor function developed concomitantly with the emergence of antigen- and mitogen-responsive T cells in patients in the acute phase of ENL. Whereas >20% suppression of PHA-P responses was seen in 7 of 12 ENL patients, only 2 of the stable LL patients showed similar levels of suppression. In general, the type of antigen used in the costimulant assay was not found to be an important factor in the generation of suppression. However, the concentration of the mitogen was important, since no difference between stable and reactional patients was observable at the optimal PHA-P concentration (200 µg/ml). Dose-related differences in levels of suppression obtained have been reported earlier with concanavalin A as a mitogen (32).

Furthermore, our ENL patients showed a relative increase in OKT8⁺ cells as indicated by the lowered OKT4/OKT8 ratio in ENL in comparison with stable LL patients. Variable results have been reported regarding T-cell subsets in ENL. Whereas some authors have reported unaltered proportions of T-cell subsets (26), others have found a reduction of the OKT8⁺ subset (15, 35). Some of these differences may be related to the stage of ENL at the time of study, ethnic differences, and methodological variables for the detection of phenotype-positive cells.

The enhanced antigen-induced T-cell reactivity observed *in vitro* was not reflected *in vivo* after concomitant intradermal challenge with soluble *M. leprae* antigens. Lack of skin test reaction was noted both during and after ENL reactions, though the same batch of antigen showed positive reactions in tuberculoid leprosy patients (mean induration = 21.75 mm). It is possible that (i) there is a time lag before the changes observed in circulation are reflected in tissues, (ii) different antigenic determinants are involved in *in vivo* and *in vitro* responses, and (iii) more than one type of delayed hypersensitivity exists in leprosy. This persistent lack of dermal reactivity is particularly intriguing, since significant increases in numbers of T4⁺ T cells have been detected in ENL lesions by us (17) and others (13), thereby ruling out any major inherent defect in the traffic of T cells into leprosy lesions.

The presence of antigen-reactive T cells in anergic LL patients has been recently shown by us (21) and others (9, 24). The emergence of T-cell reactivity, albeit transiently, in

ENL patients is a further indication that *M. leprae*-responsive T cells are present in unresponsive LL. ENL appears to be a natural model for the study of immunological perturbations that occur against a background of LL. Histological evidence of bacillary fragmentation in ENL lesions suggests that these transient immunological phenomena are of biological significance. Whether immunoregulatory phenomena, immune complexes, or altered or hidden antigenic determinants of *M. leprae* precede the development of T-cell reactivity is unclear, since it was not feasible to study the patients before the development of clinical signs. However, systematic follow-up of the natural course of disease in reactional patients and the dissection of the immunological mechanisms that are responsible for the emergence of antigen-reactive T cells in LL would have far-reaching implications for the understanding and treatment of this disease.

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