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Type 1 Reactions in Leprosy—Heterogeneity in T-cell Functions Related to the Background Leprosy Type[1]

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Leprosy, caused by *Mycobacterium leprae*, is one of the best examples of human disease in which host immune responses play a dominant role in determining the wide spectrum of clinical types seen in most populations ([25]). The stable, paucibacillary tuberculoid (TT) and the multibacillary lepromatous (LL) types of leprosy are at the extreme poles. The majority of leprosy patients belong to the less-stable borderline forms of the disease which have been defined on the basis of their near similarity to either of the polar forms, i.e., borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL) leprosy ([30]). Overwhelming evidence suggests that T-cell responses correlate with protective immunity in leprosy ([6, 15]), although the precise mechanisms influencing the antigen-specific anergy in lepromatous leprosy are still under investigation ([8, 11, 19, 26, 27]).

In addition, leprosy patients suffer from "reactions" which are acute, episodic inflammatory states unrelated to secondary infection and occurring during the natural course of the disease. The nomenclature for the various types of reactions has been confusing in the past ([23]), but it is now generally agreed that most reactions fall into a) type 1 (reversal reaction) occurring in BT, BB, and BL patients and b) type 2 or erythema nodosum leprosum (ENL) associated with polar (LL) and borderline lepromatous (BL) leprosy ([9]). The former is less defined and is generally limited to the lesions, neighboring nerves, and is unaccompanied by systemic symptoms. Microscopically, the lesions show the consistent presence of dermal edema, and some influx of T cells ([12, 14]) followed later by fibroblast proliferation and epithelioid cell whorls ([24]). Type 1 reactions may be followed by worsening or improvement of the disease, resulting in regrading of the clinical type within the leprosy spectrum. Type 2 or ENL reactions, on the other hand, are associated with fever, joint pains, and crops of raised erythematous dermal nodules which histologically show evidence of vasculitis ([32]), immune complex deposition ([1, 13]), and increase in T cells with the helper phenotype Leu3a ([14]).

Little information is available on the mechanisms underlying leprosy reactions ([2]). In earlier years, ENL was reported to be associated with immune complex deposition ([1, 13, 21, 32]). Recent evidence from our laboratory ([10]) as well as others ([22, 31]) has, in addition, indicated perturbation of T-cell functions during ENL reactions. Immunologically, type 1 reactions have been difficult to define. Increased delayed-type hypersensitivity (DTH), enhanced lymphoproliferation to *M. leprae* antigens ([4, 7]), and plasma factors ([3]) have been reported earlier.

Since these episodic clinical exacerbations during the natural course of the disease may help in the understanding of the immune defect in leprosy, we have been systematically studying T-cell-related, antigen-specific responses and immunoregulatory phenomena in patients undergoing reactions ([10]). The present investigation undertaken on borderline leprosy patients indicates that type 1 reactions reflect heterogenous immunological phenomena related to T cells. Many of the paucibacillary responder BT patients during reactions showed a deterioration in T-cell function; whereas the multibacillary, nonresponder BB and BL patients showed improvement in *in vitro* T-cell reactivity. As expected, these changes are temporary and are not reflected in the antigen-induced 48-hr skin-test reactivity which continues to reflect the initial leprosy classification.

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MATERIALS AND METHODS

Patients. Seventy-three borderline leprosy patients classified on the basis of clinical, bacteriological, and histopathological evaluation as per the Ridley-Jopling scale ([25]) were included in the study. Further subdivision of the patients was done as follows: a) The tuberculoid group included 15 nonreactional (BT) patients and 19 patients in type 1 reaction (BTR). b) The borderline group included 11 borderline (BB) and 9 borderline lepromatous (BL) patients and 7 BB and 12 BL patients with type 1 reaction (BR).

The immunological evaluations were done: a) during the active reactional phase, prior to initiation of antireaction therapy. The antireaction therapy consisted of aspirin, clofazimine (200 mg or 300 mg daily) and prednisolone (20–60 mg daily) in addition to antileprosy drugs (100 mg dapsone daily, 1200 mg rifampin monthly); and b) 4–6 months after subsidence of reaction and completion of antireaction therapy.

Stimulants. Phytohemagglutinin (PHA-P; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) was used at suboptimal (20 μg/ml) and optimal (200 μg/ml) concentrations (as tested on control subjects) for the lymphoproliferative assay and only the suboptimal concentration for the suppressor-cell assay ([10]).

Armadillo-derived *M. leprae* used at a concentration of 5×10^6 bacilli per million leukocytes in the leukocyte migration inhibition test (LMIT) was kindly provided by Dr. R. J. W. Rees, National Institute for Medical Research, London.

Integral *M. leprae* obtained from biopsies from highly bacilliferous lepromatous leprosy patients as described earlier ([20]) were used at 5×10^5, 5×10^6, and 5×10^7 bacilli/ml for the lymphopholiferative assay. Only the optimal responses are included in the results. Five million bacilli/ml were used for the suppressor-cell assay, since a majority of the responder tuberculoid patients showed optimal lymphoproliferative responses at this concentration (data not shown).

Sonicated *M. leprae* (10^8 bacilli/ml) were prepared in a Branson sonifier cell disruptor model B-30 (Branson Sonic Power Co., Danbury, Connecticut, U.S.A.) at 50 hertz for 30 min. The bacillary suspension was maintained at melting-ice temperature during the entire period. Dilutions corresponding to bacterial concentrations described for integral *M. leprae* were prepared in RPMI 1640 medium (GIBCO Laboratories, Grand Island, New York, U.S.A.), and filtered through 0.45 μm membranes (Millipore Corp., Bedford, Massachusetts, U.S.A.) before use in the lymphoproliferative and suppressor-cell assays.

Leprosin A: Sonicated armadillo-derived *M. leprae* antigens at a protein concentration of 10 μg/ml were obtained from Dr. R. J. W. Rees, and 0.1 ml was injected intradermally for assessment of *in vivo* delayed-type hypersensitivity (DTH) reaction.

Leukocyte migration inhibition test (LMIT). The LMIT was performed as described earlier ([10]). In brief, leukocytes obtained from heparinized venous blood (10 IU preservative-free heparin; Microlabs, Bombay, India) were washed twice and exposed to *M. leprae* antigens at 37°C for 1 hr in an atmosphere of humidified 5% CO_2 in air. The cells were charged into 20 μl capillaries (Arthur H. Thomas Co., Philadelphia, Pennsylvania, U.S.A.). Control cell suspensions were subjected to the same treatment in the absence of antigen. The capillaries were cut below the cell supernate interphase after centrifugation at 200 rpm for 2 min, and the cells were allowed to migrate for 16 hr at 37°C in migration chambers (Laxbro, Pune, India) containing RPMI 1640 medium and 10% fetal calf serum (Microlabs). The migration index (MI) was calculated as:

$$\frac{\text{area of migration with antigen}}{\text{area of migration without antigen}}$$

Lymphoproliferation assay. The separation of peripheral blood mononuclear cells (PBMC) ([5]) and details of the assay were as reported earlier ([10]). In brief, quadruplicates of 10^5 PBMC in 100 μl of medium containing 10% autologous plasma were exposed to 25 μl of: a) medium, b) PHA-P at 20 μg/ml, c) PHA-P at 200 μg/ml, d) integral *M. leprae* at 5×10^5/ml, e) integral *M. leprae* at 5×10^6/ml, f) integral *M. leprae* at 5×10^7/ml, and g), h), and i) sonicated *M. leprae* at concentrations corresponding to the three integral antigen concentrations in round-

TABLE 1. *Clinical evaluation of reactional patients.*

Clinical signs	BTR[a]	% Patients affected	BR[b]	% Patients affected
No. of lesions	1–3	100	Multiple	100
Erythema/induration	2+–3+[c]	100	1–3+	100
Hypoesthesia	Lesional	94	Lesional	94
Scaling	1+–2+	21	1+	21
Nerves thickened	2+–3+	73	2+–3+	84
Nerves tender	2+–3+	37	±–2+	31
No. of nerves involved	1–9	73	1–8	84
Fever	—	—	1–2+	21
Joint pains	—	—	1–2+	31
Edema	—	—	1–2+	15

[a] Borderline tuberculoid (BT) patients in type 1 reaction.
[b] Borderline (BB)-borderline lepromatous (BL) patients in type 1 reaction.
[c] 1+ = mild; 2+ = moderate; 3+ = severe.

bottomed microculture plates (NUNC, Roskilde, Denmark) for 72 hr (mitogen cultures) or 144 hr (antigen cultures) in an atmosphere of 5% CO_2 and air at 37°C. At 18–20 hr prior to harvesting of the cells by a semiautomatic cell harvester (Ilacon, Tonbridge, U.K.), they were pulsed with 0.5 μCi of ^{3}H-thymidine (specific activity, 1.80 Ci/mmol; Bhabha Atomic Research Centre, Trombay, India), and the incorporated radioactivity counted in a Rackbeta II 1217 scintillation counter (LKB Instruments, Inc., Rockville, Maryland, U.S.A.). The degree of proliferation was expressed as the stimulation index (SI) which was calculated as:

$$\frac{\text{mean counts per minute (cpm) in stimulated cultures}}{\text{mean cpm in unstimulated cultures}}$$

Suppressor-cell assay. Quadruplicates of 10^5 PBMC in 100 μl were exposed to 25 μl of: a) medium, b) integral or sonicated *M. leprae* (5×10^6/ml), c) integral or sonicated *M. leprae* followed by 25 μl of PHA-P (20 μg/ml) 24 hr later, d) 25 μl PHA-P (20 μg/ml) followed by pulsing with 0.5 μCi of ^{3}H-thymidine as described above. Percent suppression was calculated as follows:

$$\frac{(\text{cpm with mitogen} - \text{cpm with mitogen-antigen})}{(\text{cpm with mitogen} + \text{cpm with antigen})} \times 100$$

Characterization of PBMC. *T cells:* Aliquots of 10^6 Ficoll-Paque separated PBMC in 100 μl medium were exposed to 5 μl of OKT3 (pan T cell) or OKT4 (helper/inducer subset) or OKT8 (suppressor/cytotoxic subset) monoclonal antibodies (Orthoclone; Ortho Diagnostics Inc., Raritan, New Jersey, U.S.A.) for 30 min at 4°C. This was followed by washing three times, and resuspension of cells in 100 μl medium. The cells were further exposed to 5 μl of fluorescein isothiocyanate-conjugated F $(ab')_2$ anti-mouse fragment (New England Nuclear Corp., Boston, Massachusetts, U.S.A.) for 60 min at 4°C. After washing as above, the cells were enumerated using a Zeiss universal microscope (Carl Zeiss, Oberkochen, Federal Republic of Germany) with epi-illumination with an HBO-50 mercury lamp, BP 450–490 excitation filter, and BP 520–560 barrier filter. A minimum of 200 cells were counted.

B Cells: PMBC (10^5) in RPMI 1640 medium were exposed to 50 μl of 1:40 fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulins (immunoglobulin IgG + IgM + IgA; Cappel Laboratories, Cochranville, Pennsylvania, U.S.A) for 60 min at 4°C. The cells were washed three times and enumerated as above.

Skin test. One-tenth ml leprosin A (10 μg/ml; soluble, armadillo-derived *M. leprae* antigen) was injected intradermally on the volar aspect of the forearm. Induration was measured 48 hr later and diameters of <10 mm were considered negative, between 10–20 mm as moderate, and >20 mm as strong reactions.

Statistical analysis. The Mann-Whitney

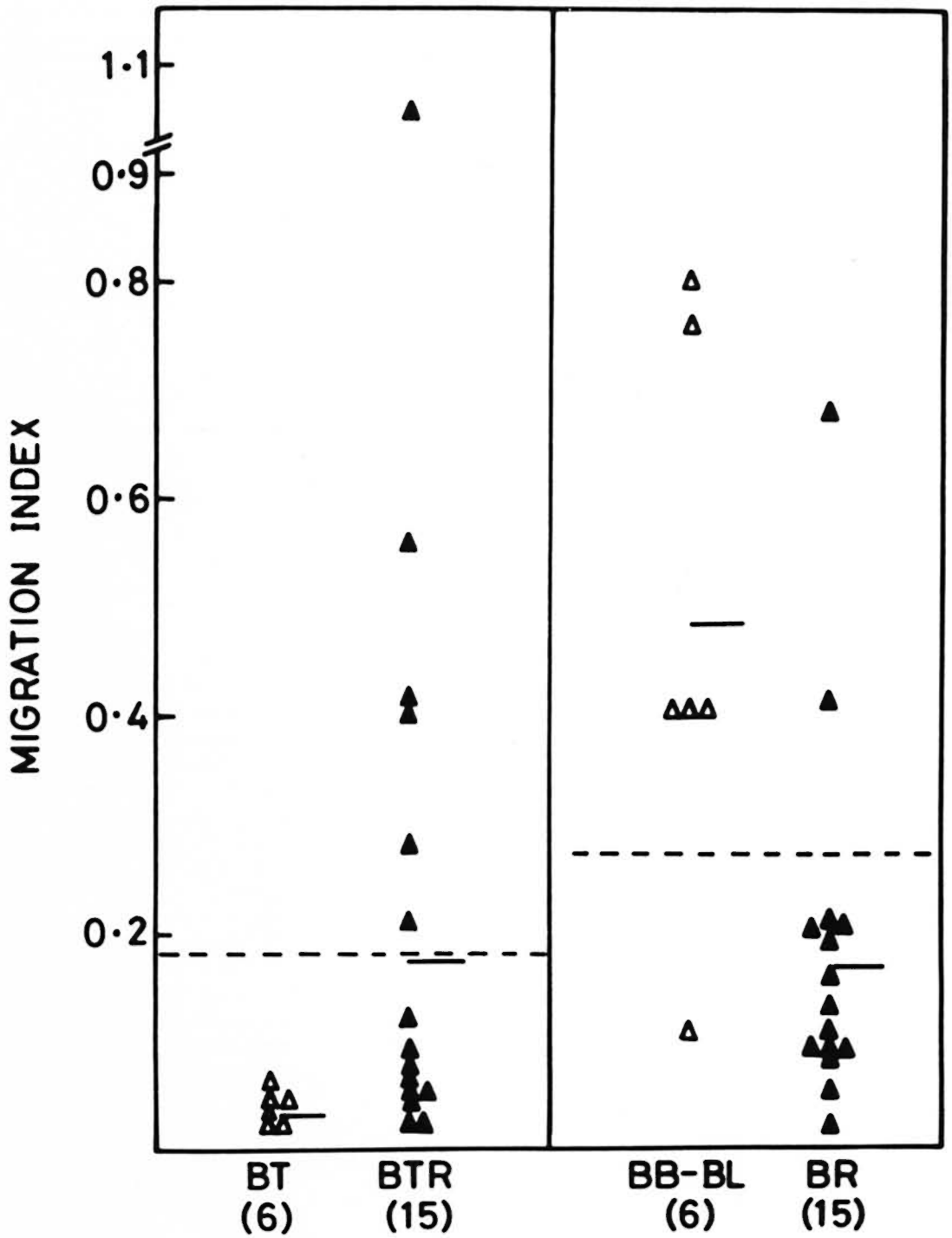

FIG. 1. Antigen-induced leukocyte migration inhibition assay showing a deterioration in 40% of BT reactional (BTR) patients in contrast to the improvement in 86% of BB-BL reactional (BR) patients as compared to the uncomplicated appropriate leprosy type. (– – –) = combined means for each group of reactional (▲) and non-reactional (△) patients of the same leprosy type as per the method of discriminant function ([29]); (——) = means of each patient group.

Migration index: Area of migration with killed integral *M. leprae*/area of migration without *M. leprae*. BT vs BB-BL, $p < 0.01$; BT vs BTR, $p < 0.01$; BB-BL vs BR, $p < 0.005$ by Mann-Whitney U test ([28]).

U test ([28]) and the method of discriminant function based on two groups ([29]) were used.

RESULTS

Seventy-three borderline leprosy patients were studied. The clinical details of 19 each of reactional BT and BB-BL patients (BTR and BR, respectively) are summarized in Table 1. The clinical grading refers to the type of leprosy diagnosed at the time of presentation. Erythema and induration of lesions and nerve tenderness were the major hallmarks of the reactional states. Histology of the BT lesions showed the expected background of epithelioid cell granulomas with prominent lymphocytic infiltration. In addition, moderate-to-severe dermal edema and extension of granulomas up to the epidermis was observed in the reactional group. The quantum of lymphocytes was considered to be within the variability noted in BT lesions, and no discernable further increase during reactional states could be observed. Acid-fast bacilli (AFB) were occasionally seen in these lesions. The BB-BL reactional lesions showed severe dermal edema and higher numbers of lymphocytes scattered among the bacilli-laden macrophages as compared to the stable BB-BL lesions.

Of the above, seven BT and eight BB-BL reactional patients were available for study 4–6 months after subsidence of clinical signs of reaction and completion of antireaction therapy.

Control nonreactional leprosy (Figs. 1–3). As expected, the control, stable paucibacillary BT patients universally showed integrity of T-cell functions as compared to the multibacillary BB-BL nonreactional subjects. Antigen-induced leukocyte migration inhibition (LMI) was significantly higher ($p < 0.01$; mean MI = 0.05 ± 0.004) in the former group as compared to the latter (mean MI = 0.48 ± 0.09). Both integral and sonicated *M. leprae* antigens were used to evaluate lymphoproliferation to surface and cytoplasmic antigens, respectively. As expected, proliferative responses were significantly higher ($p < 0.01$) in BT than in BB-BL patients.

Similar results were obtained when the general T-cell mitogen PHA-P was used at both optimal and suboptimal concentrations (Table 3). Delayed-hypersensitivity skin tests to a sonicated preparation of armadillo-derived *M. leprae* (leprosin A) showed measurable 48-hr skin erythema and induration in BT (range: 12–30 mm) as compared to the multibacillary patients who showed universally negative skin tests (<10 mm).

In continuation with our and other studies ([10, 11, 15]), immunoregulatory events related to suppressor T cells were studied in these patients using a costimulant assay wherein the effect of *M. leprae* antigens on mitogen responses was quantified. As reported earlier by us ([10, 18]), the tuberculoid patients with integrity of T-cell functions showed higher suppression ($p < 0.01$) of PHA responses by both integral and sonicated *M. leprae* as compared to the hyporesponsive/anergic BB-BL patients (Fig. 3). However, the enumeration of cells bearing phenotypic markers for the helper-inducer (OKT4) and suppressor/cytotoxic (OKT8) T-cell subsets showed no significant differences between the responder BT and nonresponder BB-BL patients during the stable form of the disease (Table 2).

Reactional borderline leprosy. Efforts were made to investigate only those reactional patients in whom the background leprosy type was unequivocal on the clinico-pathological grading of Ridley and Jopling ([25]). The borderline leprosy type 1 reactional patients were divided into those having the borderline tuberculoid (BTR) or borderline borderline-borderline lepromatous (BR) types of leprosy.

The status of the various T-cell parameters in the reactional patients was compared with the nonreactional control subjects using a cut-off boundary point for each parameter based on the method of discriminant function ([29]).

Leukocyte migration inhibition (LMI). BT patients in type 1 reaction showed heterogenous responses. Six out of 15 reactional patients showed a deterioration of antigen-induced LMI; the rest showed values similar to the stable BT patients (Fig. 1).

In contrast, the reactional BB-BL patients (13/15) showed an improved migration index (mean MI = 0.16 ± 0.14) which was highly significant ($p < 0.001$) as compared to the hyporesponsive control subjects.

Lymphoproliferation, *M. leprae* antigens (Fig. 2). The cut-off point for the stimula-

TABLE 2. *Lymphocyte subsets in peripheral blood mononuclear cells of reactional and nonreactional leprosy patients.*

Leprosy type	Mean ± S.D. of cells characterized			Ratio OKT4/OKT8
	OKT4+	OKT8+	SIg+[a]	
Stable BT N = 16	48.96 ± 12.5	44.93 ± 8.6	16.6 ± 0.96	1.1 ± 0.18
Reactional BT N = 9	55.5 ± 8.5	46.78 ± 9.3	20.0 ± 0.68	1.26 ± 0.43
Stable BB-BL N = 6	44.8 ± 9.17	33.6 ± 8.8	21.2 ± 1.01	1.38 ± 0.33
Reactional BB-BL N = 9	48.72 ± 8.03	46.56 ± 11.28	25.3 ± 1.1	1.14 ± 0.46

[a] SIg+ = surface immunoglobulin positive cells are B cells.

tion index (SI) in the BT and BTR patients was 4.26 and 3.93 for lymphoproliferation induced by integral and sonicated *M. leprae*, respectively. During the reactional phase, BT patients showed significantly lower antigen-induced lymphoproliferation ($p < 0.05$); 10/10 and 9/10 had a SI below the cut-off point for integral and sonicated bacillary antigens, respectively.

Sixty percent of the hitherto anergic BB-BL patients, on the other hand, showed significant improvement in antigen-induced lymphoproliferation ($p < 0.05$) during the reactional episodes. In three individuals the degree of lymphoproliferation reached the levels observed in the responder stable BT patients (Fig. 2).

Phytohemagglutinin (PHA-P). Both optimal and suboptimal concentrations of PHA-P were used to assess general T-cell proliferation in order to detect dose-related changes, if any, during the reactional states (Table 3). The mean Δcpm ± S.E.M. of the control and reactional groups showed no differences when the higher concentration of mitogen was used. However, the tuberculoid reactional patients (BTR) showed significantly lower lymphoproliferation as compared to the stable group ($p < 0.05$) on stimulation with suboptimal concentration of PHA-P.

Suppressor-cell activity (Fig. 3). In an attempt to assess suppressor-cell activity, the effect of integral and sonicated *M. leprae* antigens on mitogen responses of patients was evaluated in a costimulant assay as described earlier ([10]). Using the cut-off boundary points (Fig. 3), it was observed that 9/10

TABLE 3. *Mean values ± standard error of ratios of T-cell subsets and T-cell functional assays in reactional and appropriate stable borderline leprosy patients.*

Parameters	BT	BTR	BB-BL	BR
PHA 5 μg/ml	7,657 ± 1,146[a]	3,952 ± 1,146[b]	2,574 ± 825	1,852 ± 479
PHA 50 μg/ml	31,383 ± 3,837	30,917 ± 7,186	15,390 ± 1,933	13,622 ± 1,384
OKT4/OKT8 ratio	1.10 ± 0.18	1.26 ± 0.13	1.38 ± 0.01	1.14 ± 0.14
Migration index	0.05 ± 0.004	0.05 ± 0.007[c] 0.45 ± 0.004[d,e]	0.48 ± 0.09	0.16 ± 0.04[f]
Stimulation index IB[g]	6.32 ± 3.60	2.4 ± 0.53[b]	1.64 ± 0.59	3.51 ± 2.76[b]
Stimulation index SB[h]	5.81 ± 3.19	2.77 ± 1.00[b]	1.7 ± 0.75	2.22 ± 0.96[b]
Percent suppression IB	57.7 ± 6.7	27.2 ± 5.57[e]	3.58 ± 3.5	36.3 ± 4.6[e]
Percent suppression SB	48.3 ± 4.9	26.2 ± 4.1[e]	0.09 ± 3.9	42.4 ± 3.26[e]

[a] Δcpm (counts per minute).
[b] $p < 0.05$, compared to nonreactional control patients, Mann-Whitney *U* test.
[c] High-responder patients.
[d] Low-resonder patients.
[e] $p < 0.01$, compared to nonreactional control patients, Mann-Whitney *U* test.
[f] $p < 0.001$, compared to nonreactional control patients, Mann-Whitney *U* test.
[g] Integral bacilli.
[h] Sonicated bacilli.

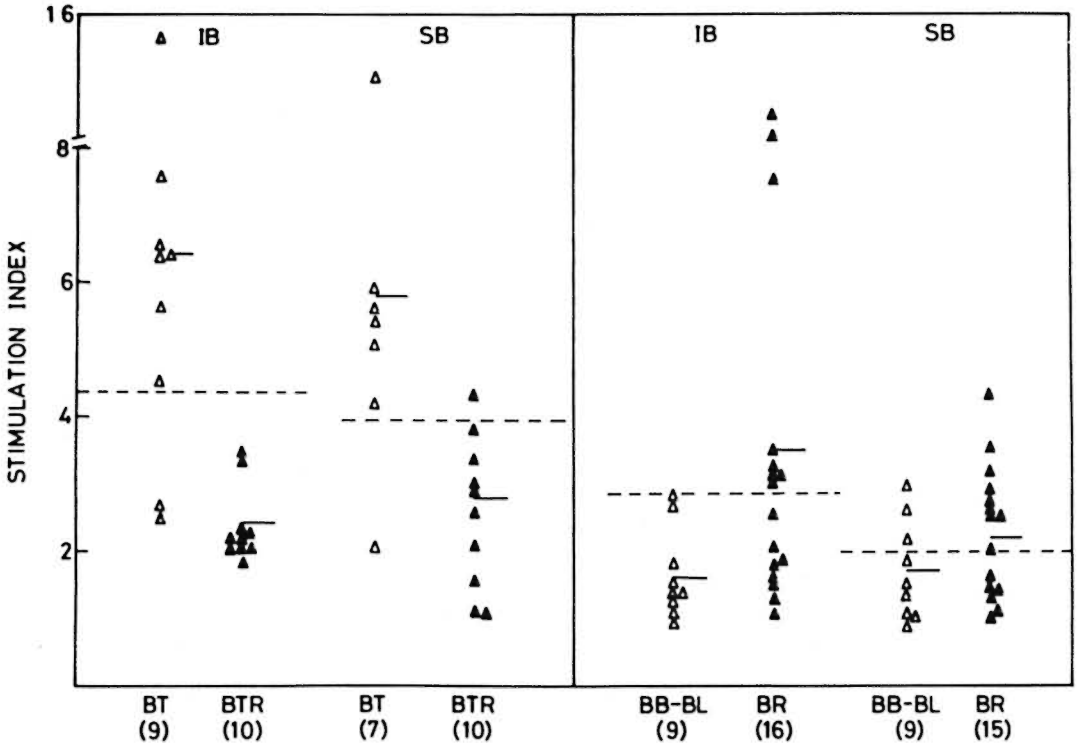

FIG. 2. Integral (IB) and sonicated (SB) *M. leprae* antigen-induced lymphoproliferation of peripheral blood mononuclear cells showing a decrease in BT reactional (BTR) patients in contrast to an increase in BB-BL reactional (BR) patients as compared to the appropriate uncomplicated leprosy type. (▲) = reactional leprosy patients; (△) = stable leprosy patients.

Stimulation index: Mean cpm of cultures with antigen/mean cpm of cultures without antigen. (– – –) and (——) same as in legend to Figure 1. BT vs BB-BL, $p < 0.01$; BT vs BTR, $p < 0.05$; BB-BL vs BR, $p < 0.05$ by Mann-Whitney *U* test ([28]).

and 7/9 patients in the tuberculoid reactional group showed lowered inducible suppressor activity ($p < 0.01$) with integral and sonicated antigens, respectively. Concurrent with improvement in T-cell-mediated leukocyte-migration inhibition and proliferation, suppressor-cell activity was also significantly enhanced ($p < 0.01$) in reactional BB-BL patients. This improvement was particularly evident with sonicated antigens, where 10/11 reactional subjects showed significant suppression of mitogen responses.

Lymphocyte subsets. The reactional tuberculoid patients did not differ from their nonreactional counterparts in the number of circulating Ig+ B cells or OKT4+ (helper/inducer) and OKT8+ (suppressor/cytotoxic) T cells (Table 3). However, the BB-BL reactional group showed a concomitant though statistically nonsignificant increase in OKT8+ cells (mean percent ± S.D. = 46.56 ± 11.2) as compared to the stable leprosy type (33.6 ± 8.8) (Table 2).

Table 3 gives the mean values and emphasizes the overall changes observed in the ratios of T-cell subsets and T-cell-related functional assays in the reactional and matched stable patients of the appropriate leprosy groups.

Delayed-type hypersensitivity (DTH) reactions. The 48-hr DTH response to soluble armadillo-derived *M. leprae* antigens was evaluated rather than the 3-wk Mitsuda reaction to integral bacilli since it was considered that the earlier response may better reflect the T-cell perturbations of the acute, transitory reactional phase (Table 4). Interestingly, in spite of the *in vitro* improvement of T-cell functions in reactional BB-BL patients, the DTH reactions continued to be negative (<10 mm).

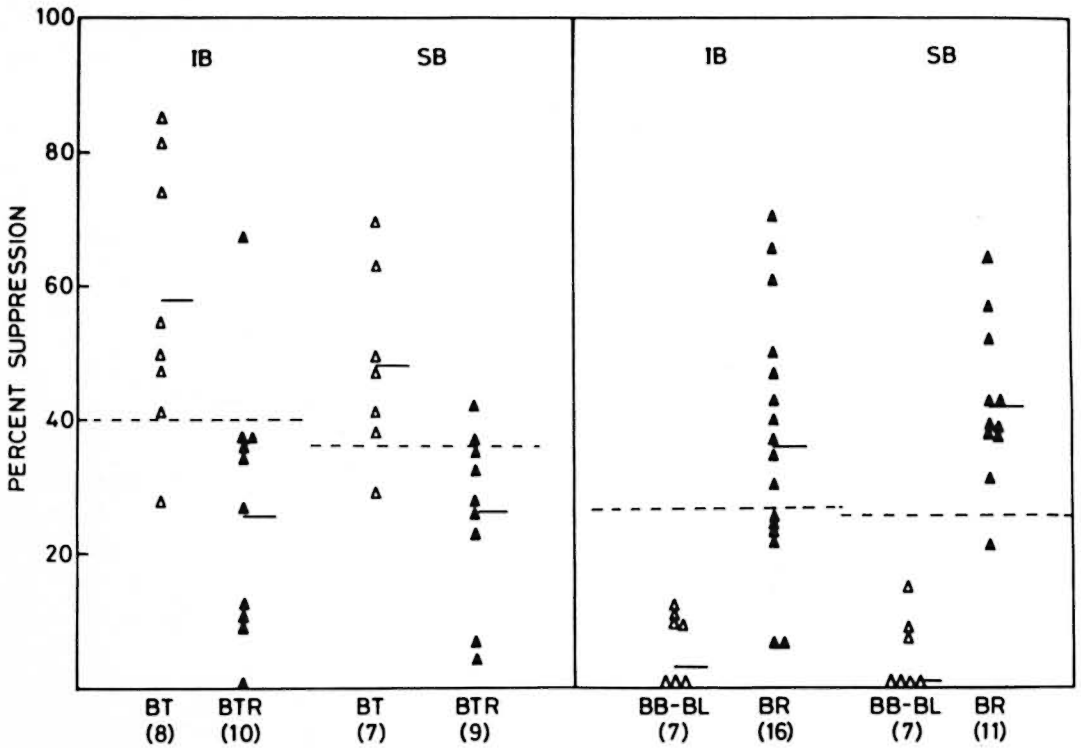

FIG. 3. Suppressor-cell activity generated by integral (IB) and sonicated (SB) *M. leprae* antigens as monitored by their effect on PHA-P-induced lymphoproliferation of peripheral blood mononuclear cells in a costimulant assay ([10]). As compared to stable BT and BB-BL leprosy patients, the reactional BT (BTR) patients showed a decrease and the reactional BB-BL (BR) patients showed an increase in antigen-induced suppressor activity.

Percent suppression: [(Mean cpm with mitogen − cpm with mitogen-antigen)/(mean cpm with mitogen + mean cpm with antigen)] × 100. (– – –) and (——) same as in legend to Figure 1. BT vs BB-BL, $p < 0.01$; BT vs BTR, $p < 0.01$; BB-BL vs BR, $p < 0.01$ by Mann-Whitney *U* test ([28]).

All except one of the tuberculoid patients in reaction showed persistence or a marginal increase in erythema and induration (20–50 mm) as compared to the stable BT group (12–34 mm). An attempt was made to correlate the skin-test reactivity with the *in vitro* T-cell functions of this group. The LMI assay correlated best with the *in vivo* state; 6/6 stable BT showed good LMI and moderate-to-strong DTH responses. Of the 11 reactional BT patients, six showed concordance for both tests and the others showed strong DTH responses but poor migration indexes. Thus, it would appear that in the reactional states of both BT and BB-BL leprosy, the skin tests of most patients reflect the background leprosy type rather than the functional perturbations in circulating T cells.

Post-reactional status (Fig. 4). Reactional patients were investigated as above 4–6 months after termination of antireaction therapy and subsidence of clinical signs of reactions. The antileprosy treatment was continued throughout the study period. In general, no consistent pattern was obtained with any of the parameters in the post-reactional phase of BT or BB-BL leprosy. Within each group, individual patients showed improvement, deterioration, or no change in leukocyte migration inhibition, lymphoproliferation, and suppressor-cell activity.

DISCUSSION

Earlier studies on type 1 reactions have been difficult to interpret due to problems in nomenclature, variability in the immunological phenomena detected, and the heterogeneity of the patient population. In most studies, BT, BB, and BL leprosy types were grouped together ([3, 4]). In view of the fact

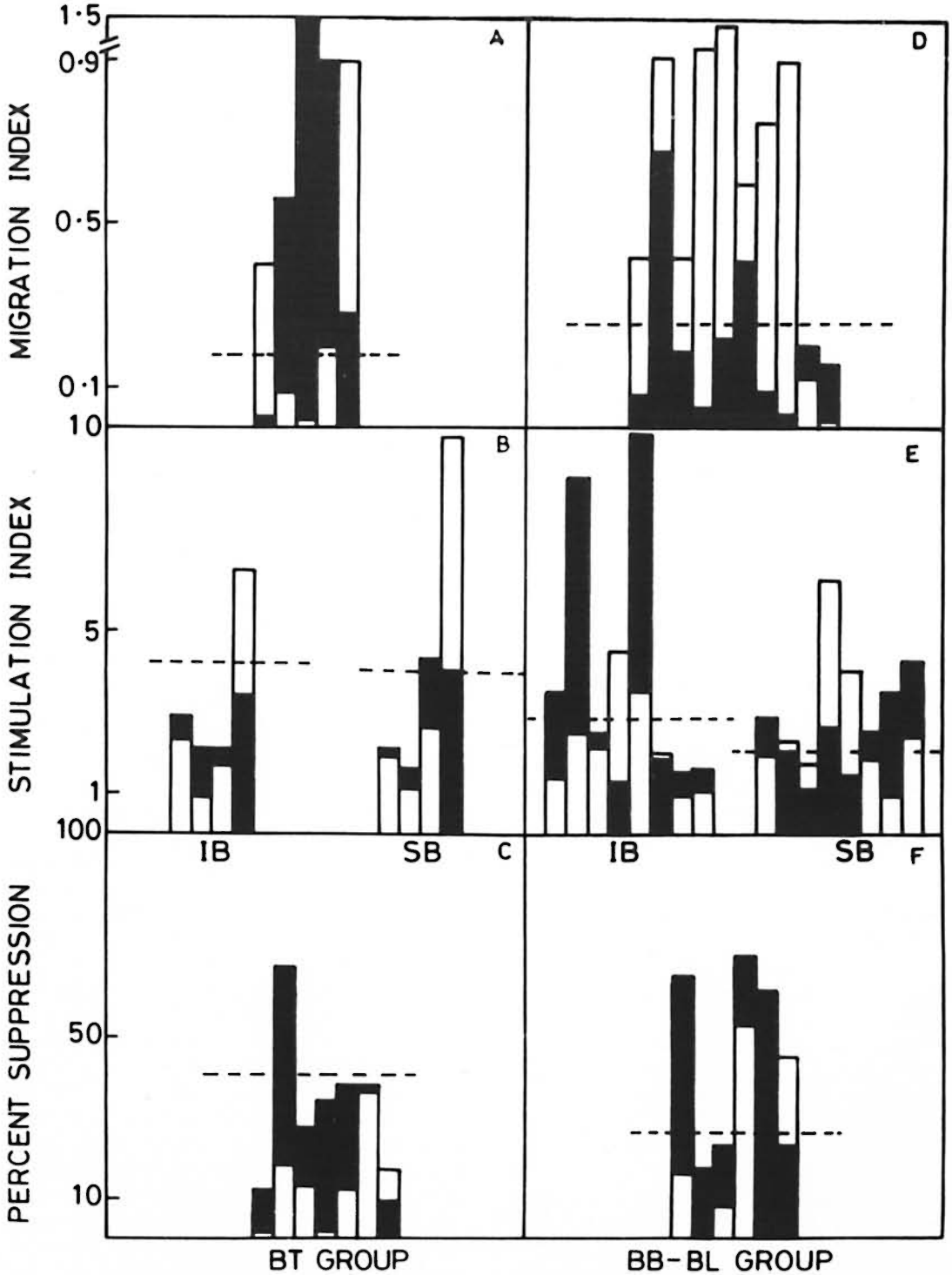

FIG. 4. Status of antigen-induced leukocyte migration inhibition (**A** and **D**), lymphoproliferation (**B** and **E**) with integral (IB) and sonicated (SB) leprosy bacilli, and suppressor-cell activity (**C** and **F**) in the same patients of BT and BB-BL leprosy during reaction (■) and 4–6 months after subsidence of reaction (□). Combined mean (– – –), migration index, stimulation index, and percent suppression were calculated as per legends in Figures 1–3.

TABLE 4. *Comparison of leukocyte migration inhibition test (LMIT) and delayed-type hypersensitivity (DTH) responses in BT nonreactional and reactional patients.*

Leprosy type	LMIT (MI)[a]	DTH (range in mm)[b]
Stable BT (6/6)	+	+
	(<0.06)	(12–34)
Reactional BT (6/11)	+	+
	(<0.09)	(20–48)
Reactional BT (5/11)	−	+
	(>0.28)	(12–50)

[a] Area of migration of leukocytes in presence of antigen/area of migration in absence of antigen.
[b] Diameter (mm) of skin induration.

that the spectrum of leprosy may influence the diversity of host responses within the reactional group, we consciously separated the patients into two broad groups: a) those who belonged to the relatively paucibacillary BT type, and b) those showing features of multibacillary leprosy of the BB-BL type. The diagnoses of the leprosy type and the reactional state were based on clinical reactions limited to lesions and neighboring nerves, absence of systemic symptoms, and histopathological evidence of reaction. The control group consisted of stable nonreactional patients of the appropriate leprosy type. In the main, T-cell functions were studied which included *in vitro* a) T-cell responses such as PHA and antigen-induced lymphoproliferation and leukocyte migration inhibition, b) enumeration of T-cell subsets with monoclonal antibodies, c) immunoregulatory events using a costimulant assay for the detection of suppressor cells as described earlier ([10, 18]), and d) *in vivo* 48-hr DTH reactions using a soluble, armadillo-derived *M. leprae* preparation (leprosin A).

Distinct differences in T-cell functions were observable between the reactional and nonreactional leprosy patients using the criteria of a) significance of less than 5% by the Mann-Whitney *U* test ([28]), as well as b) cut-off point for each parameter as defined by the method of discriminant function ([29]). The percentage of peripheral blood T cells and T-cell subsets, on the other hand, did not show any significant differences between the two groups of both leprosy types.

The present investigation draws attention to the varied T-cell responses that occur during the reactional phase of leprosy. The predominant finding was the dichotomy in T-cell perturbations that occurred in the tuberculoid and borderline lepromatous reactional patients. The stable BT subjects had the expected integrity of T-cell functions, while a significant number of the reactional patients showed deterioration of antigen-induced leukocyte migration inhibition, lymphoproliferation, and suppressor-cell activity. It is intriguing that with the T-cell parameters used, differential effects were observed. Ninety percent of BT reactional patients showed deterioration of lymphoproliferative responses; whereas 40% showed lowered LMI responses. Interestingly, an earlier study had reported enhanced blast transformation in BT patients showing clinical downgrading ([7]). In confirmation of our earlier reports ([10, 16, 18, 19]), stable BT patients showed normal or enhanced suppressor-cell activity; whereas 90% of the reactional patients had reduced suppression of PHA responses in the concurrent presence of *M. leprae* antigens. In spite of the deterioration in the functional status of circulating T cells, the antigen-induced DTH responses tested on the same day continued to be sustained or even enhanced.

In contrast, in the hyporesponsive/anergic leprosy (BB, BL) the acute phase of type 1 reaction was uniformly associated with the emergence of antigen-reactive T cells in the blood as judged by the enhanced antigen-induced lymphoproliferation in 50%–60% and leukocyte migration inhibition in 86% of the reactional subjects. In agreement with our earlier studies ([10, 16, 18, 19]), suppressor-cell activity was poor in the nonreactional BL patients; whereas it was enhanced in reactional BB-BL subjects. Interestingly, sonicated bacillary antigens suppressed PHA responses in more individuals (10/11) than integral heat-killed bacilli (10/16). This antigenic difference was not reflected in the lymphoproliferative responses of our Indian patients, as was earlier suggested on the Ethiopian population who had an association of nerve damage with enhanced lymphoproliferation induced by sonicated *M. leprae* ([4]).

It would appear from the present study that the clinical nomenclature of type 1 does

not adequately reflect on the nature of the background immunological perturbations that occur during the reactional episodes in the two forms of leprosy. The T-cell changes that occur in type 1 reactions are the converse of the basic immune status of the background leprosy type, i.e., many BT patients show a decrease and BL patients show a uniform enhancement of T-cell functions. Thus, the high-responder BT patients and the low-responder BB-BL patients during the acute phase of type 1 reaction show similar levels of immune responsiveness to antigens and mitogens (Table 3). This results in an apparent and parodoxically similar level of immune responsiveness in the responder and nonresponder leprosy type during the reactional phase. Moreover, in conformity with our earlier studies on type 2 or ENL reactions in lepromatous leprosy, anergic borderline leprosy patients also showed a natural emergence of antigen-reactive T cells in the circulation ([10]) and the entry of T cells with helper phenotype into their hitherto lymphopenic lesions ([14]).

These results on reactional BB-BL patients further reinforce our earlier views that a) suppressor-cell activity reflects T-cell integrity and parallels a healthy T-cell response in leprosy, and b) many anergic patients possess antigen-reactive T cells ([10, 17]) and may benefit clinically from suitable strategies using immunological modulation of the host response to *M. leprae*. Moreover, it would appear that the background leprosy type influences the level of T-cell reactivity during reactions. Whether this is related to the unmasking of antigenic determinants, modulation by antigen-antibody complexes in the bacilliferous forms of leprosy, or other immunoregulatory events is not clear. Our previous studies have shown a uniform increase in T6+ Langerhans' cells in the epidermis of reactional skin which may attribute a primary or secondary role to these antigen-presenting accessory cells.

A limitation of the present study is the single point follow-up that was possible in our patients. The studies of the post-reactional phase showed marked individual variability in the T-cell responses and did not help to explain either the mechanisms behind the transitory nature of the observed changes or the final clinical outcome of the disease. Further studies are required to investigate whether the lack of expression of skin-test reactivity in the hyporesponsive patients in the presence of detectable T-cell functions in blood and T-helper cells in lesions is time related or influenced by other local factors.

SUMMARY

Nineteen each of paucibacillary borderline tuberculoid (BT) and multibacillary borderline borderline (BB)/borderline lepromatous (BL) leprosy patients undergoing type 1 reactions were compared with nonreactional stable patients of the appropriate leprosy type. In the BT reactional group, both phytohemagglutinin-induced and, more importantly, antigen-induced lymphoproliferation was reduced in 80%–90% of the patients. On the other hand, leukocyte migration inhibition was reduced in 40% and remained unchanged in the others. Suppressor-cell activity as evaluated by a costimulant assay was also reduced in a majority of the reactional BT individuals. In contrast, the bacilliferous BB and BL patients in reaction showed significant general improvement in leukocyte migration inhibition ($p < 0.001$) and antigen-induced lymphoproliferation ($p < 0.05$) as compared to the expected hyporesponsive/anergic uncomplicated BB-BL patients. Suppressor-cell activity also recovered during the reactional phase. However, no significant differences were observed in either of the reactional or stable leprosy types in the numbers of total T cells (OKT3+) and their subsets as defined by OKT4+ (helper/inducer) and OKT8+ (suppressor/cytotoxic) functional phenotypes. Moreover, during type 1 reactions the 48-hr delayed-type hypersensitivity (DTH) responses after intradermal injection of *Mycobacterium leprae* antigens continued to reflect the background leprosy type rather than the functional perturbations in the circulating T cells. Only a marginal increase in DTH was observed in some BT reactional individuals. No consistent pattern in the above *in vitro* T-cell-related responses was discernable in the same individuals 4–6 months after subsidence of reactions. The clinical entity of type 1 reactions encompassing paucibacillary and multibacillary leprosy shows a heterogeneity/dichotomy in T-cell responses which may reflect different im-

munological mechanisms underlying the reactional state.

RESUMEN

Se estudiaron 19 pacientes con lepra tuberculoide (BT) paucibacilar y 15 pacientes con lepra intermedia (BB)/intermedia lepromatosa (BL) multibacilar, con reacciones en piel del tipo 1, y los resultados se compararon con los encontrados en pacientes estables no reaccionales del tipo correspondiente. En el grupo reaccional BT, la linfoproliferación inducida por fitohemaglutinina y, más importantemente, por el antígeno, estuvo reducida en el 80 al 90% de los pacientes. Por otro lado, el índice de inhibición de la migración de leucocitos estuvo reducido en un 40% de los pacientes y permaneció sin cambio en los otros. La actividad de las células supresoras evaluada por un ensayo de coestimulación también estuvo reducida en la mayoría de los pacientes BT reaccionales. En contraste, los pacientes baciliferos BB y BL reaccionales mostraron una significante mejoría general en la inhibición de la migración de leucocitos ($p < 0.001$) y en la linfoproliferación inducida por antígeno ($p < 0.05$) en comparación con los pacientes hiporespondedores/anergicos de los tipos BB-BL sin complicaciones reaccionales. La actividad de las células supresoras también se recuperó durante la fase reaccional. Sin embargo, no se observaron diferencias significativas entre los grupos reaccional o estable de pacientes con lepra en cuanto al número total de células T (OKT3+) y sus subclases OKT4+ (cooperador/inductor) y OKT8+ (supresor citotóxico). Además, durante las reacciones del tipo 1, las respuestas de hipersensibilidad retardada (HTT), 48 horas después de la inyección de los antígenos del *Mycobacterium leprae*, continuaron reflejando más bien el tipo basal de lepra que las perturbaciones funcionales en las células T circulantes. Sólo se observó un aumento marginal de la HTT en algunos individuos BT reaccionales. Sin embargo, no se observó un patrón consistente en las respuestas *in vitro* relacionadas con las células T en los mismos individuos, 4–6 meses después de que las reacciones cedieron. Las características clínicas de las reacciones del tipo 1 en los casos paucibacilares y multibacilares de la lepra, muestran una heterogeneidad en las respuestas de las células T, lo cual puede reflejar diferentes mecanismos inmunológicos característicos del estado reaccional.

RÉSUMÉ

On a comparé, d'une part, 19 malades atteints de lèpre paucibacillaire tuberculoide dimorphe (BT) et 19 malades présentant une lèpre multibacillaire dimorphe dimorphe (BB) ou lépromateuse dimorphe (BL), les uns et les autres présentant des réactions de type 1, avec d'autre part des malades ayant une forme stable de lèpre du type approprié, mais non réactionnel. Dans le groupe réactionnel BT, on a observé une réduction de 80 à 90% à la fois dans la production provoquée de phytohémagglutinine et, ce qui est plus important, dans la prolifération des lymphocytes induite par des antigènes. Par ailleurs, l'inhibition de la migration des leucocytes a été réduite de 40% alors qu'elle restait inchangée chez les autres. L'activité des cellules du type suppresseur, évaluée par un essai avec co-stimulant, était également réduite chez la majorité des individus présentant une lèpre réactionnelle BT. Par contre, les malades bacillifères BB et BL en réaction présentaient une amélioration générale significative par l'épreuve d'inhibition de la migration des leucocytes ($p < 0{,}0001$) et celle de la prolifération des lymphocytes induite par des antigènes ($p < 0{,}05$), par rapport aux valeurs auxquelles on aurait du s'attendre si on les compare aux réponses caractéristiques de l'anergie chez les malades atteints de lèpre BB-BL non compliquée. L'activité des cellules du type suppresseur retournait cependant à la normale au cours de l'épisode réactionnel. Néanmoins, aucune différence significative n'a été observée, tant chez les malades réactionnels que chez ceux atteints de lèpre stable, quant au nombre total de cellules T (OKT3+) ou à leurs sous-groupes, à savoir les phénotypes définis au point de vue fonctionnels comme OKT4+ (cellules adjuvantes/inductrices) et OKT8+ (cellules du type suppresseur/cytotoxique). De plus, au cours des réactions de type 1, les réponses d'hypersensibilité retardée (DTH) notées 48 heures après l'injection intra-dermique d'antigènes de *Mycobacterium leprae* continuaient à refléter le type de lèpre sous-jacent plutôt que les perturbations fonctionnelles dans les cellules T circulantes. On n'a observé qu'une augmentation marginale de l'hypersensibilité de type retardé, et ce, chez quelques individus réactionnels BT. Aucun profil cohérent n'a pu être mis en évidence chez les mêmes individus, 4 à 6 mois après la disparition des réactions, dans les profils des réponses *in vitro* des cellules T considérées ci-dessus. L'entité clinique constituée par les réactions de type 1, qui couvre à la fois la lèpre paucibacillaire et la lèpre multibacillaire, présente une hétérogénéité et une dichotomie dans les réponses des cellules T qui reflètent peut-être les différents mécanismes immunologiques qui sont sous-jacents à l'état réactionnel.

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