Clin. exp. Immunol. (1984) **55**, 623–628.

Differences in predominant T cell phenotypes and distribution pattern in reactional lesions of tuberculoid and lepromatous leprosy

R. B. NARAYANAN,* S. LAAL,† A. K. SHARMA‡, L. K. BHUTANI† & INDIRA NATH*
*Departments of *Pathology and †Dermatology, All-India Institute of Medical Sciences and ‡Safdarjung Hospital, New Delhi, India*

(Accepted for publication 21 October 1983)

SUMMARY

The nature and histological pattern of the cutaneous infiltrates of 17 leprosy patients in reversal reactions (Type I) and erythema nodosum leprosum (Type II, ENL) were compared with tissues from 18 non-reactional borderline leprosy (BT, BL) and lepromatous leprosy (LL) patients using monoclonal antibodies and immunofluorescence. Reactional BT lesions showed a mild increase in OKT11$^+$ pan T cells as compared to non-reactional tissues and a significant influx of OKT8$^+$ (suppressor/cytotoxic) cells which were peripherally localized in the lymphocyte mantle surrounding the epithelioid cells. The Leu 3a$^+$ (helper/inducer) cells were scattered amongst the lymphocytes and macrophages. The mean ratio ($\pm$s.d.) of Leu 3a$^+$/OKT8$^+$ cells was $1{\cdot}88 \pm 0{\cdot}64$ in Type I BT reactions as compared to $2{\cdot}95 \pm 0{\cdot}95$ in BT lesions. In contrast, lesions of BL reversal reactions and ENL showed a more marked increase in pan T cells with a preponderance of the helper/inducer subset, Leu 3a$^+$/OKT8$^+$ ratio being $2{\cdot}26 \pm 0{\cdot}61$ and $0{\cdot}93 \pm 0{\cdot}57$ in BL reactional and non-reactional lesions, respectively. Interestingly, this increase in the numbers of the T cells reached levels observed in BT lesions. The distribution pattern of OKT8$^+$ cells was similar to Leu 3a$^+$, both being diffusely scattered amongst the bacilli laden macrophages. Ia like antigens were present in all granulomas and were abundant on lymphocytes and macrophages and less conspicuous on epithelioid cells. T6$^+$ Langerhans cells were uniformly increased in all reactional lesions. It would appear that the changes observed in both Type I and Type II reactions are similar in the lepromatous group of patients. They differ significantly from the BT reversal reaction in terms of the dominant T cell subset and the microanatomical distribution of the OKT8$^+$ cells in the lesions.

Keywords T cell phenotypes leprosy reactions

INTRODUCTION

In leprosy, host immune responses play a dominant role in determining the clinical types of the disease with the localized tuberculoid leprosy (TT) and the generalized lepromatous leprosy (LL) forming the two poles of a five point spectrum. The borderline, intermediate forms of leprosy (BT, BB, BL) are associated with unstable immunity and a varying clinical picture (Nath, 1983; Ridley & Jopling, 1966; Turk & Bryceson, 1971). In addition, 20–25% of patients with leprosy undergo episodic reactions which are accompanied by systemic manifestations and tissue injury.

Correspondence: Dr Indira Nath, Department of Pathology, A.I.I.M.S., New Delhi-110029, India.

Erythema nodosum leprosum (ENL, Type II) is a well characterized reaction which occurs predominantly in polar LL patients who develop multiple, inflammatory nodules, joint pains and fever. The other commonly occurring reversal reaction (Type I) is seen mainly in borderline leprosy (BT, BB, BL), and presents with edematous and erythematous lesions against the background leprosy type. Less frequently, reactions localized to active lesions and devoid of systemic manifestations may also be seen. The immunological perturbations underlying the various reactions are ill understood. ENL has been thought to be due to immune complexes deposited in the tissues (Wemambu *et al.*, 1969; Bjorvatn *et al.*, 1976). Reversal reactions on the other hand may reflect an exacerbated delayed type hypersensitivity reaction (DTH, Bjune *et al.*, 1976; Bjune, 1983), sometimes followed by upgrading of the clinical picture towards TT.

Sites of ENL lesions show foci of neutrophils against a mononuclear cell background and occasional vasculitis (Job, Gude & Macadem, 1964), whereas reversal reactions show increased dermal edema, nerve damage and other evidences of DTH (Ridley, 1977). Recently, our laboratory (Narayanan *et al.*, 1983) as well as others (Modlin *et al.*, 1983; Van Voorhis *et al.*, 1982) had shown the utility of monoclonal antibodies (MoAbs) defining T cell subsets in understanding the immunopathology of leprosy lesions. Differences in both the numbers and the distribution pattern of functional T cells was observed in non-reactional TT and LL. Cells with helper/inducer phenotypes were diffusely scattered amongst the epithelioid cells and lymphocytes, and those with suppressor/cytotoxic markers were observed in a ring like manner in the lymphocyte cuff of tuberculoid lesions. In the present study, using similar antibodies and immunofluorescence, skin biopsies from patients with ENL and reversal reactions were studied with a view to further understanding the cellular dynamics of the cutaneous infiltrates of the active reactional lesions.

MATERIALS AND METHODS

Skin biopsies. Three to five millimetre size biopsies were obtained from typical lesions from 35 leprosy patients (six BT, six BT in reversal [Type I] reaction), four BL, five BL in reversal reaction, eight polar LL and seven LL with ENL [Type II] reaction). The patients were graded on the clinicopathological criteria of Ridley & Jopling (1966) and Ridley (1977). Reactional states were diagnosed as ENL when LL patients presented with fever, joint pains and subcutaneous erythematous nodules, as reversal reactions when borderline leprosy patients presented with nerve tenderness and erythematous plaques. Tissue specimens were divided into portions for histological diagnosis on conventional paraffin sections and for immunofluorescence on quick frozen tissues stored at −20°C upto 72 h.

Immunofluorescence. Five micrometre thick serial cryostat sections (IEC, USA) were cut at −20°C, air dried and fixed in an acetone:chloroform mixture (1:1) and exposed to the following MoAbs for 30 min at room temperature. OKT11 (pan T cell), OKT6 (subset of T cells and Langerhan's cells), OKT8 (suppressor/cytotoxic T cells) and OKIa were obtained from Ortho Pharmaceutical Corporation, Raritan, New Jersey, USA. Leu 3a (helper/inducer T cell) was kindly donated by Becton Dickinson, USA. All sections were rinsed in 0·5 M phosphate-buffered saline pH 7·2 (PBS) for 30 min and exposed to FITC conjugated sheep antimouse $F(ab)_2$ (New England Nuclear, Boston, Massachusetts, USA) mixed with 1% pontachrome violet for another 30 min at room temperature. For the definition of B cells, direct immunofluorescence was done using FITC conjugated rabbit anti-human IgM (Dakopatts A/S, Denmark). All the sections were once again rinsed in PBS, mounted in 10% glycerol-PBS containing paraphenylene diamine and viewed by epi-illumination using a HB50 mercury lamp and Zeiss universal microscope.

Specificity controls consisted of staining of serial sections with omission of primary antibody. Optimal dilutions of antibodies and the checking of primary antibody was done on cryostat sections of lymph nodes obtained at surgery and on peripheral blood mononuclear cells of healthy volunteers.

The cytological nature of the cellular infiltrates and bacillary content was evaluated on serial cryostat sections and conventional sections stained with haematoxylin and eosin (H&E) and

Zeihl–Neelson stains. Quantitation of subsets of cells in typical well formed granulomas was done by enumeration of positive cells with green fluorescence and negative cells staining red with pontachrome violet. The number of Langerhans cells were quantitated per 100 keratinocytes.

RESULTS

Reversal reactions (Type I)

Six BT and five BL patients were diagnosed to be in reversal reactions as assessed clinically and by histology of the lesions.

BT

As expected, BT skin biopsies showed epithelioid cell granulomas surrounded by lymphocytes. The corresponding reactional tissues had in addition dermal edema with some granulomas encroaching the epidermis. Such lesions showed a marginal increase in $OKT11^+$, pan T cells and a significant influx of Leu $3a^+$ (helper/inducer) and $OKT8^+$ (suppressor/cytotoxic) T cells (Fig. 1, Table 1). The ratio of Leu $3a^+/OKT8^+$ cells showed a relative increase in suppressor/cytotoxic phenotype in reactional BT, as compared to non-reactional lesions. The $OKT8^+$ cells were mainly distributed in the peripheral lymphocyte mantle surrounding the granuloma, whereas Leu $3a^+$ cells were admixed with other lymphocytes and scattered singly amongst the central epithelioid cells.

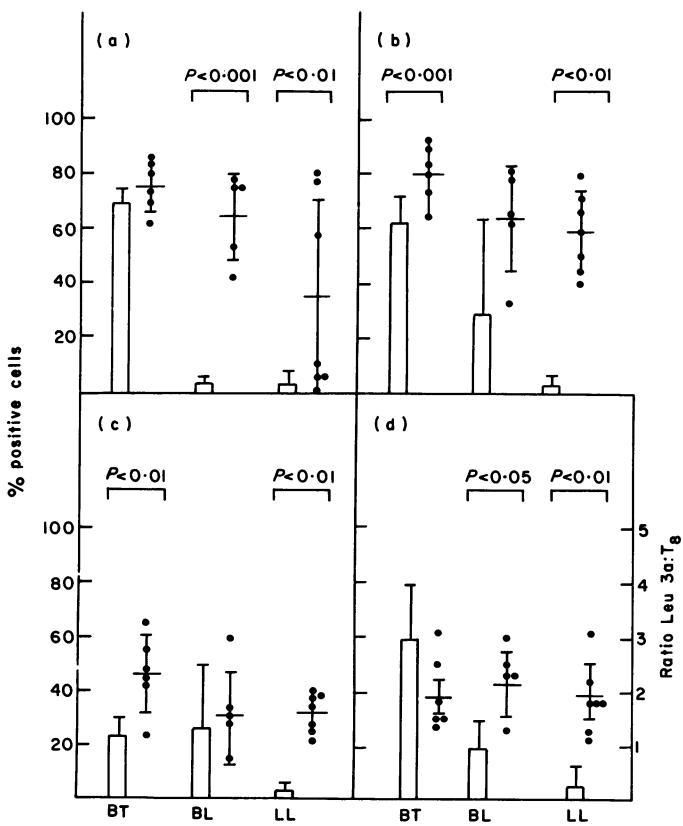

Fig. 1. Percentage of T cells and their subsets in dermal granulomas of leprosy patients with and without reactions as defined by MoAbs and indirect immunofluorescence (a = Pan T; b = Leu 3a; c = OKT8). Ratio of helper/inducer (Leu $3a^+$) to suppressor/cytotoxic ($T8^+$) cells is shown in (d). The bars indicate % positive cells (mean ± s.d.) in non-reactional BT ($n = 6$), BL ($n = 4$) and polar LL ($n = 8$) with the corresponding scattergram showing individual values in lesions of BT and BL patients in reversal reactions and lepromatous patients with ENL. ⊣⊢ indicates mean ± s.d., n = number.

Table 1. Percentage of cells reactive with MoAbs in the lesional skin of leprosy patients during reversal reactions (Type I) and ENL (Type II)

	Reactional states	Mean ± s.d. dermal granulomas						Ratio	Mean ± s.d. Langerhans cells
		OKT11	Leu 3a	OKT8	OKT6	OKIa	IgM*	Leu 3a/OKT8	(OKT6)
I	Reversal BT ($n=6$)	75·32 ± 9·07	79·9 ± 10·37	46·1 ± 14·04	4·0 ± 3·0	98·5 ± 2·1	—	1·88 ± 0·64	24·0 ± 3·0
	BL ($n=5$)	64·4 ± 15·97	63·8 ± 18·78	31·2 ± 17·40	3·0 ± 3·0	100	1	2·26 ± 0·61	30·0 ± 5·0
II	ENL ($n=7$)	34·6 ± 36·20	58·8 ± 14·91	32·7 ± 7·48	—	98·5 ± 1·4	1	1·87 ± 0·66	27·0 ± 5·0
								Normal skin ($n=5$)	16·0 ± 2·0
								BT + BL + LL ($n=17$)	17·0 ± 3·0

* Heterologous anti-human IgM antibody.
n = number of patients.

BL

The granulomas of BL reversal reactions showed a highly significant increase in OKT11$^+$ as well as an increase in Leu 3a$^+$ and OKT8$^+$ cells (Fig. 1, & Table 1). The quantum of T cells and their subsets reached levels comparable to that seen in stable BT lesions. The ratio of Leu 3a$^+$/OKT8$^+$ indicated a greater proportion of cells with helper/inducer phenotype as compared to non-reactional lesions of the same leprosy type. The OKT8$^+$ as well as Leu 3a$^+$ cells were distributed diffusely and scattered singly or in small clusters amongst the bacilli laden macrophages.

ENL (Type II reactions)

Skin biopsies from seven patients of polar LL having ENL reactions as determined by subcutaneous erythematous nodules, fever and joint pains were studied. Histologically, the skin nodules had neutrophilic aggregates over a background of foamy histiocytes and granular bacilli. Though lymphocytic infiltration was not as obvious in the H&E stained sections, cryostat sections showed a significant increase in OKT11 pan T cells in three biopsies. Both Leu 3a$^+$ and OKT8$^+$ cells were also increased and their ratios showed a preferential increase in helper/inducer cell types (Fig. 1 & Table 1), similar to what was observed in BL reversal reactional tissues. The distribution was random with scattering of small clusters of T cells amongst the bacilli containing macrophages.

B cells

There was no increase in B cells in any of the lesions ($< 1\%$) as characterized by anti-human IgM antibody.

Cells with T6 and Ia like antigens

The Langerhans cells in the epidermis stained intensely for T6 and Ia like antigens. They showed uniform increase in lesions of ENL, BT and BL in reversal reactions, as compared to normal skin and their respective non-reactional counterparts (Table 1). A few T6$^+$ cells (3–4%) were also seen in dermal granulomas or reversal reactions but not in ENL lesions. Ia positive cells formed the predominant cells in all lesions. Ia$^+$ positivity was associated with lymphocytes and macrophages but was less conspicuous with epithelioid cells.

DISCUSSION

Reactional states in leprosy are confusing and difficult to define in terms of immunological mechanisms. Though the clinical and histological features of ENL are distinct, more subtle changes are associated with reversal reactions (Ridley, 1977). The present study using MoAbs defining functional subsets of lymphocytes, macrophages and Langerhans cells was directed at understanding the immunopathological changes occurring in reactional lesions as compared to non-reactional granulomas of the same leprosy type. Such studies are inherently limited by: (i) the fact that the antibodies define only a phenotypic marker, albeit of functional subsets of cells; (ii) the semi-quantitative nature of the enumeration of cells in histological sections and (iii) the single time observation of a dynamic process. Nevertheless, increase in pan T cells, differences in the type of dominant T cell subset and the distribution pattern of $OKT8^+$ cells in the reactional tissues of tuberculoid as compared to lepromatous lesions was impressive and difficult to ignore.

B cells appeared to be relatively uninvolved, though small numbers of plasma cells were sometimes seen in BL lesions. Ia like antigens were abundant and associated with macrophages, T cells and Langerhans cells irrespective of the presence or absence of reactions. T6 antigen was associated with increased Langerhans cells in the epidermis of all reactions as well as with some cells in the dermal infiltrates of borderline leprosy reactions. $T6^+$ cells were not observed in the dermal lesions of ENL.

Significant differences were noticed between the BT and BL groups of patients undergoing reversal reactions. Whereas T cells were increased in both types of lesions, the increase was more impressive in the reactional BL, where the T cell influx reached levels comparable to the numbers seen in localized tuberculoid leprosy. Though the ratios of Leu $3a^+$/$OKT8^+$ cells were similar in the various reactions (Table 1), differences in the dominant T cell phenotype became evident on comparison with the control non-reactional leprosy types (Fig. 1). Thus, cells with suppressor/cytotoxic phenotype were proportionally increased in BT as compared to helper/inducer phenotype in BL reactions. ENL lesions showed features similar to BL. In addition, the pattern of distribution of T cell subsets was different in the reactional lesions of the tuberculoid and lepromatous patients. In Type I reactions of BT, $OKT8^+$ cells maintained the peripheral 'ring' like location seen in the non-reactional lesions of tuberculoid leprosy (Modlin *et al.*, 1983; Narayanan *et al.*, 1983). In contrast, reactional BL and ENL showed random admixture of macrophages with bacilli, Leu $3a^+$ and $OKT8^+$ cells.

It is interesting that the dominant T cell subset and the microanatomical pattern of the cellular infiltrates differed in the tuberculoid and lepromatous lesions irrespective of the type of reaction. That ENL and reversal reactions of BL which were thought to have a different genesis should show similar T cell types and pattern suggests a need for the reevaluation of the role of T cells in these disorders. Whether the microenvironment required for and the traffic pattern followed by the helper/inducer and suppressor/cytotoxic cells is different during the course of various types of immunoinflammation is not known. It is possible that the histological pattern of the two subsets of T cells in leprosy lesions may represent differences between successful and unsuccessful local immunity. They may explain the subsequent limited and temporary improvement commonly observed after reactions in LL as compared to the more effective self-limiting nature of tuberculoid leprosy. Such studies may have importance in the evaluation of the nature and duration of immunological improvement associated with immunotherapy or immunoprophylaxis. Since T cells are associated with effective immunity in leprosy, it is encouraging to note that natural reactions result in the influx of T cells into lepromatous leprosy, indicating the presence of reactive T cells in the hitherto non-responding leprosy type.

This work was supported by the British Leprosy Relief Association (LEPRA). R.B. Narayanan was a Pool Officer of Council of Scientific and Industrial Research (CSIR). Mr Maheshanand rendered technical assistance.

REFERENCES

BJORVATN, B., BARNETSON, R.ST.C., KRONVALL, G., ZUBLER, R.H. & LAMBERT, P.H. (1976) Immune complexes and hypercatabolism in patients with leprosy. *Clin. exp. Immunol.* **26,** 388.

BJUNE, G. (1983) Reactions in leprosy. *Lepr. Rev.* (special issue), **June 1983,** 61 S.

BJUNE, G., BARNETSON, R.ST.C., RIDLEY, D.S. & KRONVALL, G. (1976) Lymphocyte transformation test in leprosy: correlation of the response with inflammation of lesions. *Clin. exp. Immunol.* **25,** 8.

JOB, C.K., GUDE, S. & MACADEN, V.P. (1964) Erythema nodosum leprosum. A clinico-pathologic study. *Int. J. Lepr.* **32,** 1977.

MODLIN, R.L., HOFMAN, F.M., MEYER, P.R., SHARMA, O.P., TAYLOR, C.R. & REA, T.H. (1983) *In situ* demonstration of T lymphocyte subsets in granulomatous inflammation: leprosy, rhinoscleroma, and sarcoidosis. *Clin. exp. Immunol.* **51,** 430.

NARAYANAN, R.B., BHUTANI, L.K., SHARMA, A.K. & NATH, I. (1983) T cell subsets in leprosy lesions: *in situ* characterization using monoclonal antibodies. *Clin. exp. Immunol.* **51,** 421.

NATH, I. (1983) Immunological aspects of human leprosy—current status. *Lep. Rev.* (Special issue), **June 1983,** 31S.

RIDLEY, D.S. (1977) *Skin biopsy in leprosy.* CIBA-GEIGY Ltd., Basle.

RIDLEY, D.S. & JOPLING, W.H. (1966) Classification of leprosy according to immunity. A five group system. *Int. J. Lepr.* **34,** 255.

TURK, J.L. & BRYCESON, A.D.M. (1971) Immunological phenomenon in leprosy and related diseases. *Adv. Immunol.* **13,** 209.

VAN VOORHIS, W.C., KAPLAN, G., SARNEE, E.N., HORWITZ, M.A., STEINMAN, R.M., LEVIS, W.R., NOGUEIRA, H., HAIN, L.S., GATTASS, C.R., ARRICK, B.A. & COHN, Z.A. (1982) The cutaneous infiltrates of leprosy: cellular characteristics and the predominant T cell phenotypes. *N. Engl. J. Med.* **307,** 1593.

WEMAMBU, S.N.C., TURK, J.L., WATERS, M.F.R. & REES, R.J.W. (1969) ENL—a clinical manifestation of the arthus phenomenon. *Lancet,* **ii,** 933.