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Normal numbers of T_6 positive epidermal Langerhans cells across the leprosy spectrum

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Summary Langerhans cells (LC) in the skin lesions of 25 untreated leprosy patients were defined by indirect immunofluorescence using monoclonal antibodies against phenotypic markers T_6 and Ia like antigens. Normal numbers of epidermal LC were seen in leprosy lesions. No differences were observed in the intensity of fluorescence or in the numbers of T_6+Ia+LC across the leprosy spectrum. However, the dermal granulomas of tuberculoid leprosy (TT/BT) showed a high proportion of T_6+cells in the mononuclear infiltrate surrounding the epithelioid cells. Smaller numbers of these cells were seen in borderline leprosy (BB, BL) with a virtual absence in polar lepromatous leprosy (LL). Ia like antigens were associated with the macrophages in BL and LL granulomas and with the lymphocytes in tuberculoid lesions. B cells were conspicuously absent in all leprosy lesions.

Introduction

Leprosy is a polymorphic granulomatous disease with a spectrum of lesions ranging from the paucibacillary epithelioid cell granulomas surrounded by abundant lymphocytes in the tuberculoid forms of leprosy to the lymphopenic lesions with bacilli laden foamy macrophages in lepromatous leprosy (LL).[1] These lesions are thought to reflect the host resistance to *Mycobacterium leprae*. The mechanisms leading to good cellular immunity in tuberculoid leprosy and the lowered T cell functions in the lepromatous form of the disease are still under investigation.[2] The immunological phenomena in leprosy have been extensively

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investigated in the past by means of *in vitro* systems using peripheral blood lymphocytes and monocytes. The *in situ* analysis of cells in leprosy lesions has been recently possible with the availability of monoclonal antibodies to phenotypic markers present on subsets of lymphocytes and monocytes.[3–5]

Accessory cells other than macrophages have been shown to play an important part in the presentation of antigen to T cells.[6,7] Langerhans cells (LC) present in the epidermis of skin have been shown to participate in experimental allergic contact dermatitis and delayed hypersensitivity reactions.[6–8] These cells bear immunological markers, in common with other macrophages, namely Fc (IgG) receptor, C_3b receptor, Ia antigens and possess small amounts of acid phosphatase and abundant ATPase enzymes.[9] They can be distinguished from morphologically similar dendritic macrophages by the presence of T_6 marker[10] which is also seen on early and activated T cells.[10,11] Scant information is available on the role of LC in the presentation of antigen in a predominantly dermal disease such as leprosy. Electron microscopic[12] and histochemical studies[13,14] indicated morphological and numerical alterations in LL. With a view to understanding the *in situ* cellular interactions in leprosy, we had earlier used indirect immunofluorescence with monoclonal antibodies directed against subsets of T cells and monocytes. Using antibodies to T_6 and Ia like antigens, we report here the results obtained on the status of Langerhans and other T_6 positive cells in the skin lesions across the leprosy spectrum.

Materials and methods

PATIENTS AND TISSUE MATERIALS

Three to five mm skin biopsies were removed from 25 untreated leprosy patients attending the Dermatology Departments of the All India Institute of Medical Sciences and Safdarjang Hospital, New Delhi. The patients were graded on the clinicopathological criteria of Ridley & Jopling.[1] Each biopsy was bisected on removal, one half was fixed in 10% buffered formalin and processed by conventional paraffin embedded blocks. The other half of the skin was quick frozen, and stored at $-20°C$. Cryostat sections were cut within 24 hours and serial sections were stained with hematoxylin and eosin (H&E), Ziehl Neelsen and monoclonal antibodies. Only those results are reported where H&E stains of formalin fixed and cryostat sections had shown typical histopathological features.[15]

IMMUNOFLUORESCENCE

Five micron thick cryostat sections (IEC, USA) were cut at $-20°C$, air dried for 5 minutes, fixed in 1:1 acetone-chloroform mixture and stained by indirect

immunofluorescence technique. Four to five serial sections were used for the antibody tested. After dipping in 50 mM phosphate buffered saline (PBS), pH 7·4, the sections were covered with 1 : 10 dilution of OKT_6 or 1 : 20 dilution of OKIa antibodies (Ortho Pharmaceutical Co., USA), and left at room temperature for 30 minutes. Control sections were covered with PBS only. Subsequently, the sections were washed in PBS for 30 minutes and layered with 1 : 60 dilution of sheep FITC conjugated antimouse $F(ab)_2$ (New England Nuclear, Boston, USA) mixed with 1 : 100 dilution of pontachrome violet for 30 minutes at room temperature. After washing as above, the sections were mounted in 90% PBS-glycerol containing paraphenylene diamine and viewed under a Carl Zeiss microscope with epillumination and HBO 50 mercury lamp. Sections of normal skin removed during surgery were used as controls.

The degrees of infiltration by lymphocytes, epithelioid cells and foamy macrophages were graded arbitrarily on serial sections stained with H&E according to the criteria of Ridley.[15] Quantitation of positive cells in: (a) typical well-formed granulomas was done by assessing the percentage of cells showing fluorescence as compared to total cells in the same field; and (b) the number of T_6 positive epidermal Langerhan cells were quantitated per 100 keratinocyte as well as per high power field.

IDENTIFICATION OF B CELLS

B cells were identified by the presence of surface IgM using direct immunofluorescence. Cryostat sections of skin were incubated with 1 : 5 dilution of normal rabbit serum for 45 minutes at 4°C and washed in 50 mM PBS pH 7·4 for another 15 minutes. They were then layered with 1 : 120 dilution of fluorescein conjugated rabbit anti-human IgM antibodies (Dakopatts A/S, Denmark) and incubated at 4°C for 30 minutes. Subsequently, the sections were washed in PBS for 45 minutes, mounted, and viewed as described above. Controls consisted of sections from human tonsils treated in a similar manner.

Results

EPIDERMIS

The number of T_6 positive epidermal dendritic cells suggestive of LC were within normal limits in the leprosy patients (Table 1). Though the lesional skin of tuberculoid patients showed an apparent increase in LC, this was not found to be statistically significant. LC enumerated per high power field or per hundred keratinocytes showed no significant differences. The intensity of fluorescence also showed no variation across the leprosy spectrum (Fig. 1). Monoclonal antibodies against T_6 identified relatively more LC than anti-Ia like antibodies (data not cited).

Table 1. Numbers of LC in the skin lesions of untreated leprosy patients

Diagnosis	Epidermis OKT$_6$ +ve cells/high power field Mean ± SE (range)
Control (Normal skin) 5*	16·0 ± 2·0 (9–21)
Tuberculoid leprosy (BT/TT) 11*	20·0 ± 2·0 (11–30)
Borderline leprosy (BB/BL) 6*	16·0 ± 2·0 (6–21)
Polar lepromatous leprosy (LL)	15·0 ± 2·0 (5–22)

Statistical analysis was done by Students 't' test.
TT, LL and BB/BL vs Control, P = not significant.
TT vs LL, P = not significant.
* Number of individuals studied.

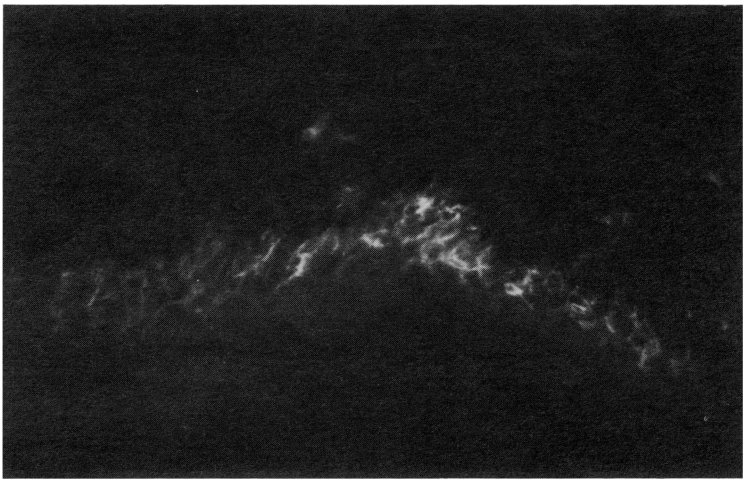

Figure 1. LC in the epidermis overlying a lepromatous lesion, showing intense immunofluorescent staining for T_6 antigen on the cell bodies and dendritic processes. (Cryostat Section; counterstained with pontochrome violet × 220.)

DERMIS

The dermal granulomas of leprosy showed cells positive for Ia like and T_6 antigens (Table 2). Ia positivity was associated predominantly with the lymphocytes surrounding epithelioid cells in tuberculoid and with foamy macrophages in lepromatous lesions as described earlier.[4] Interestingly, T_6 positive cells lacking dendritic processes reminiscent of LC were found in the lymphocyte mantle of

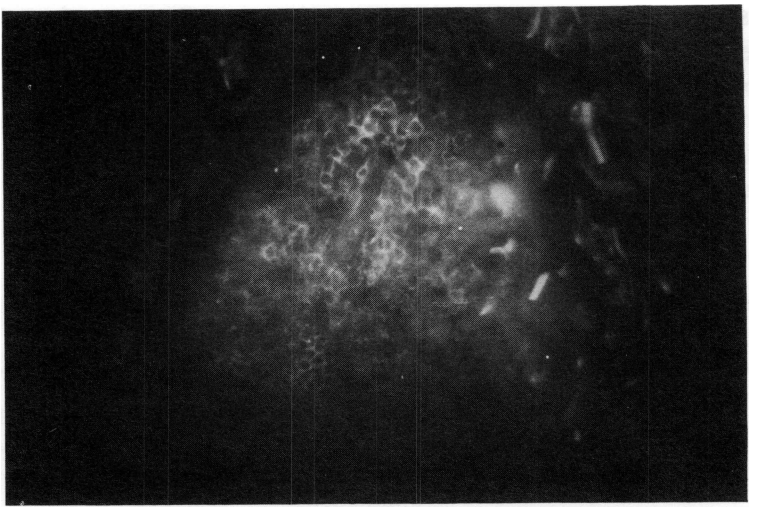

Figure 2. Dermal granuloma of tuberculoid leprosy showing immunofluorescent staining for T_6 antigen on small round nondendritic cells in the lymphocyte mantle surrounding small collections of epithelioid cells. (Cryostat Section; counterstained with pontochrome violet $\times 220$.)

Table 2. Numbers of OKT_6 and OK Ia positive cells in the dermal lesions of patients with tuberculoid leprosy

	Histopathology			Infiltrating cells in the dermal granuloma (range percentage positive cells)		
Diagnosis	Lymphocytes	Epithelioid cells	AFB	T_6	Ia	B cells (surface IgM)
BT	++	++	—	50–60	80–90	ND
BT	+	+	—	30–40	80	ND
BT	+	+	—	5–10	70–80	ND
BT	+	+	—	5–10	80	±
BT	+	+	—	5–10	70–80	±
BT	+	+	—	5–10	90	ND
BT	++	++	—	ND	80–90	ND
BT	+	+	—	ND	80–90	ND
TT	++	++	—	70–80	80–90	ND
TT	++	++	—	40–50	90	ND
TT	++	++	—	30–55	90–95	ND

± = occasional; ND = not detectable.

TT/BT lesions (Fig. 2). Smaller numbers of such cells were also observed in borderline leprosy (Table 3). Lesions of lepromatous leprosy showed a conspicuous lack of T_6 positive cells but had abundant Ia + T_6 − macrophages (Table 4).

No detectable staining was observed with anti-human IgM antibodies in leprosy granulomas indicating the absence of B cells. These antibodies had shown intense staining for B cells in the human tonsils and lymphnodes.

Table 3. Numbers of OKT_6 and OK Ia positive cells in the dermal lesions of patients with borderline leprosy

	Histopathology			Infiltrating cells in the dermal granuloma (range percentage positive cells)		
Diagnosis	Lymphocytes	Macrophages	AFB	T_6	Ia	B cells (surface IgM)
BB	±	+	1+	5–10	100	ND
BB	+	+	2+	5–10	100	ND
BB	+	+	1+	ND	100	ND
BL	+	+	3+	5–10	100	not done
BL	+	+	3+	5–10	100	ND
BL	±	+	3+	ND	100	ND

± = occasional; ND = not detectable.

Table 4. Numbers of OKT_6 and OK Ia positive cells in the dermal lesions of patients with polar LL

	Histopathology			Infiltrating cells in the dermal granuloma (range percentage positive cells)		
No.	Lymphocytes	Macrophages	AFB	T_6	Ia	B cells (surface IgM)
1	±	++	$5\frac{1}{2}$+	ND	100	ND
2	±	++	$5\frac{1}{2}$+	ND	100	ND
3	−	++	6 +	ND	100	ND
4	±	++	6 +	ND	100	ND
5	±	++	$5\frac{1}{2}$+	ND	100	not done
6	−	++	6 +	ND	100	ND
7	±	++	6 +	ND	100	ND
8	±	++	6 +	ND	100	ND

ND = not detectable.

Discussion

Functional studies involving cells from the lesional tissues face operational problems. Thus attempts were made in the present study to evaluate the status of antigen presenting cells by means of monoclonal antibodies directed against functional phenotypic markers. No significant differences were found in the numbers of LC in the lesional skin across the leprosy spectrum though an apparent increase was observed in TT/BT. In particular, LL patients known to have depressed T cell responses showed normal levels of these cells. However, these studies do not rule out qualitative and degradative changes in LC as reported by previous workers using other methodologies.[13,14] Where ATPase has been used as a marker of LC, it is possible that differences in the content of this enzyme may explain the reported apparent reduction of these cells in LL.

The dermal granulomas of many leprosy patients showed both Ia+ and T_6+ cells. In TT/BT lesions T_6+ cells appeared to be nondendritic small cells scattered amongst the lymphocytes surrounding the epithelioid cells. Though it is likely that LC have migrated from the epidermis to the dermal granuloma, the T_6 marker occurs on the immature T cells in the thymus.[11] Therefore, it is possible that the T_6 positive cells in the dermis are of T cell lineage. Our earlier studies[4] had shown these areas to have lymphocytes which were predominantly Ia+T_3+(pan T cell) with a large proportion of T_4+ (helper/inducer) and to a lesser extent T_8+(suppressor/cytotoxic) cells.

The presence of normal numbers of LC and the abundance of Ia like antigens on macrophages of LL would suggest that the antigen presenting capacity of these 2 accessory cells may not be significantly defective. It would seem therefore that the paucity of T cells in the granulomas may be the major cellular defect leading to non-killing of bacilli within the macrophages.

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