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Parasitism of antigen presenting cells in hyperbacillary leprosy

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SUMMARY

Full thickness skin biopsies from four patients with borderline lepromatous leprosy (BL leprosy) have been examined. Immunohistological techniques have been employed to analyse the non-lymphoid mononuclear cells present in the dermal infiltrates associated with the BL lesions. This analysis was performed using three monoclonal antibodies, RFD2 (recognizing macrophages), RFD1 (recognizing interdigitating cells) and NA1/34 (recognizing Langerhans cells). It was found that the vast majority of non-lymphoid mononuclear cells in the lesions were RFD2$^+$ macrophages. However, a significant number (15–30%) of macrophage like cells were RFD1$^+$ interdigitating cells. A very small number of NA1/34$^+$ Langerhans cells were also identified within the dermal infiltrates. Combination immunohistology and Ziehl Neelsen staining revealed that all these cell types could be found containing the *Mycobacterium leprae* organisms. The proportions of parasitized cells within each subpopulation was equivalent to the overall proportion of each cell type within the infiltrate. The significance of parasitism of cell types thought to be involved in antigen presentation and induction of immune responses is discussed.

Keywords leprosy macrophages parasitism antigen presenting cells

INTRODUCTION

Macrophage–lymphocyte interaction is recognized as a fundamental mechanism within acquired immunological responses (Unanue, 1981). As *Mycobacterium leprae* is a facultative intramacrophage parasite, the infection of these cells might be expected to compromise the immunological defence network of the host. However, despite the fact that hyperbacillary forms of leprosy are almost inevitably associated with host anergy, the persistent infection of macrophages is thought to be the result rather than the cause of immunological unresponsiveness. This is perhaps due to the fact that the macrophages are viewed primarily as effector cells (Convit *et al.*, 1974), and it is concluded that their ineffectiveness in removing the *M. leprae* stems from an underlying immunological defect (Godal *et al.*, 1972).

It is known however that the macrophages can act as antigen presenting cells (APCs), being inducers of T lymphocyte responses (Unanue 1981). Is this function then being compromized by *M. leprae* infection? Furthermore, macrophages are not alone in the role of antigen presenting cells. Langerhans cells have been shown to act as antigen presenting cells (Silberberg-Sinakin *et al.*, 1978) and are inevitably present in the skin. Interdigitating (ID) cells although normally located in the T

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lymphocyte areas of lymph nodes and spleen (Heusermann, Strutte & Muller-Hermelink, 1974; Hoefsmit *et al.*, 1980) are also thought to act as antigen presenting cells and have been shown to be present in many chronic inflammatory reactions (Poulter *et al.*, 1982, 1983b, Mishra *et al.*, 1983). It is not yet established whether these cells are involved (along with macrophages) in the dermal infiltrates associated with leprosy.

However, the recent development of immunohistological techniques using monoclonal antibodies (MoAbs) that recognise these accessory cells has made it possible to identify them within tissue sections (for review see Poulter, 1983).

The use of MoAbs to 'stain' specific cell populations and subpopulations has already contributed to a more precise understanding of the histopathology of leprosy (Narayanan *et al.*, 1983; Modlin *et al.*, 1983). These workers used immunohistological techniques to analyse the microenvironment of leprosy skin lesions, with special emphasis on the disposition of the lymphocyte populations. In the present paper new MoAbs are used to identify macrophages, Langerhans cells and interdigitating cells. The study focuses on material from hyperbacillary borderline lepromatous leprosy (BL leprosy), as it was felt relevant to determine the relationship between these immunocompetent accessory cells and the mycobacteria. The study has two aims. Firstly, to determine whether the macrophage like cells in BL lesions are a homogeneous population and secondly, to determine whether the mycobacteria infect only one type of cell, or can parasitize various cell types.

MATERIALS AND METHODS

Clinical material. Full thickness skin biopsies were taken from four patients with clinically and histopathologically defined BL leprosy.

Preparation of tissue sections. Biopsy material was embedded in OCT medium and frozen in isopentane cooled in a bath of liquid nitrogen. Six micrometre sections were cut on a cryostat at −25°C, air dried for 1 h and fixed for 5 min in a 1:1 mixture of chloroform–acetone.

Immunohistology. Indirect immunofluorescence or immunocytochemical methods were performed as fully described previously (Mason *et al.*, 1983; Poulter *et al.*, 1983a). The MoAbs used and their specifications are listed in Table 1. In some studies reagents were combined to determine cellular interrelationships. In these cases immunoperoxidase and immunoalkaline phosphatase methods were used sequentially with separate first layer reagents; or immunofluorescence methods were used combining two first layer MoAbs of different immunoglobulin class which were then visualized with mixtures of two class specific second layer reagents conjugated to fluorescein isothiocyanate and rhodamine tetraethyl thiocyanate.

Cell phenotyping. To establish the phenotypic characteristics of macrophages, interdigitating cells and Langerhans cells, immunohistological examination of control tissues was performed

Table 1. MoAbs used in this study

Designation	Class	Source	Specificity
RFDR2	IgM	Dept. Immunology R.F.H.	HLA-DR antigens
RFD1	IgG1	Dept. Immunology R.F.H.	Interdigitating cells 10% subpopulation of B lymphocytes
RFD2	IgG1	Dept. Immunology R.F.H.	Monocytes/macrophages
NA1/34	IgG2	A. McMichael Oxford	Langerhans cells Cortical thymocytes

where morphology and location could be used to confirm the identity of the individual cell types. The results of these studies are presented in Table 2.

Ziehl–Neelsen (ZN) staining. In some studies immunoalkaline phosphatase methods were followed by ZN staining for mycobacteria.

Analysis. Multiple fields of at least two and up to six preparations of each immunohistological 'stain' were examined for each specimen. Quantitation of the proportion of parasitized cells of each phenotype was performed by counting numbers of parasitized and non-parasitized cells after combined staining with RFD1/ZN, RFD2/ZN and NA1/34/ZN.

The proportions of individual cell populations were estimated by eye (see footnote to Table 3).

RESULTS

Histopathology

Sections from all biopsies were stained with haematoxylin & eosin and with ZN stain. Extensive mononuclear cell infiltration was seen in the dermis, predominantly composed of macrophage like cells. Lymphocytes were seen distributed diffusely throughout the infiltrates. The ZN stain revealed multiple mycobacteria in clumps of macrophage like cells throughout the dermis. No obvious organization of the granulomata was seen.

Identification of macrophages, interdigitating cells and Langerhans cells

It can be seen from Table 2 that each of these cell types expressed a distinctive phenotype when examined in normal tissues. More specifically, it was found that these three cell types (macrophages, interdigitating cells and Langerhans cells) could be identified one from another using only three MoAbs: RFD2 recognizing macrophages, RFD1 recognizing interdigitating cells and NA1/34 recognizing Langerhans cells. Studies of the leprosy material therefore concentrated on the use of these three MoAbs.

The presence of RFD1$^+$ cells

Using the immunoalkaline phosphatase method significant numbers of RFD1$^+$ cells were observed in the dermis of all specimens. These cells constituted 15–30% of the non-lymphoid cells in the infiltrate. This proportion varied from one area of the section to another and it was difficult to identify precisely individual cells due to the interdigitating morphology causing cell membranes to closely interpose (Fig. 1). Subsequent ZN staining demonstrated that a proportion of RFD1$^+$ cells contained mycobacteria (Fig. 1 & Table 3).

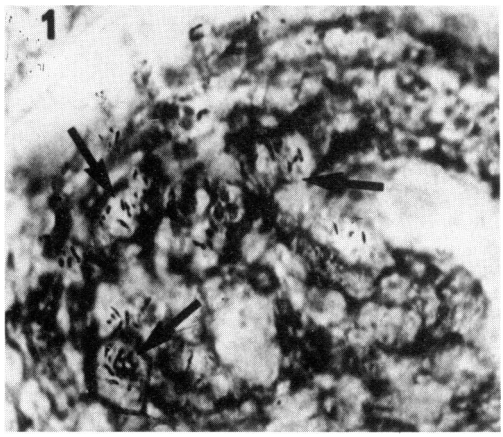
Table 2. The phenotyping of non-lymphoid accessory cells in normal tissues

Cell type	Tissue	Location	Morphology	RFDR2	RFD1	RFD2	NA1/34	ACP†	ATP‡
Interdigitating cells	Tonsil	interfollicular paracortex	mononuclear large dendritic interdigitating	+	+	−	−	−	+
Macrophages	Tonsil	Marginal zone, diffuse distribution in follicles and medulla	mononuclear round/dendritic	+/−*	−	+	−	+	+/−
Langerhans cells	Normal skin	Epidermis	mononuclear interdigitating elongated	+	−	−	+	−	+

* +/− = some cells +ve some cells −ve.

† ACP = Cytochemical reactions for lysosomal acid phosphatase activity.

‡ ATP = Cytochemical reactions for membrane adenosine tri-phosphatase.



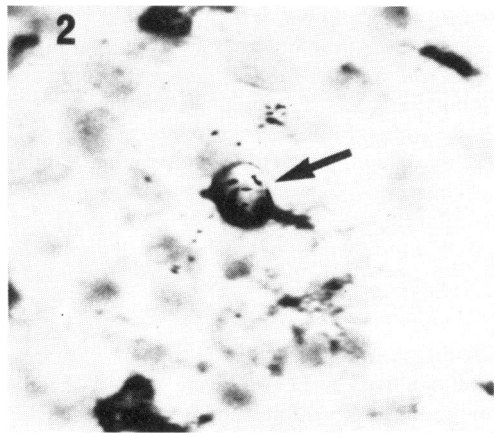


Fig. 1. A cluster of RFD1$^+$ ID cells in the dermis of a patient with BL leprosy. ID cells are identified using the immunoalkaline phosphatase method. Following fixation in buffered formalin and washing in Tris-buffered saline a ZN stain was performed on the same section. A proportion of RFD1$^+$ cells clearly contain *M. leprae* (arrows). Magnification × 630.

Fig. 2. A Langerhans cell containing *M. leprae* in the dermis of a patient with BL leprosy (arrow). Langerhans cells were identified using the MoAb NA1/34. *M. leprae* were identified in the same sections with a ZN stain. (Method details as for Fig. 1). Magnification × 630.

The presence of NA1/34$^+$ cells

Immunohistological staining with MoAb NA1/34 demonstrated significant numbers of Langerhans cells in the epidermis. The appearance of NA1/34$^+$ cells in the dermis was rare. In some sections no positive cells were found in the dermis while in others quite distinct clumps of NA1/34$^+$ cells occurred, particularly around hair follicles (Fig. 2). These cells never constituted more than 1% of the macrophage like cells in the dermis. NA1/34 immunohistology was also combined with ZN

Table 3. Presence and parasitism with *M. leprae* of RFD1$^+$, RFD2$^+$ and NA1/34$^+$ cells in BL leprosy skin lesions

Cell type	% of mononuclear non-lymphoid cells*	% of all cells parasitized that expressed each specific phenotype
Macrophages RFD2$^+$	70–80†	70–90
Interdigitating cells RFD1$^+$	15–30	10–30
Langerhans cells‡ NA1/34$^+$	0–2	0–1

* Summation of RFD1$^+$, RFD2$^+$ and NA1/34$^+$ cells was taken as 100% non-lymphoid mononuclear cells.

† These ranges represent an estimate of the proportion of all non-lymphoid mononuclear cells that were expressing the phenotype of a particular subpopulation. Two to six sections from each biopsy were reacted with each MoAb. Multiple fields were examined on each section.

‡ In the dermis only.

staining. Scanning of all sections stained in this way (> 10 sections) resulted in the observation that some of the NA1/34$^+$ cells in the dermis contained *M. leprae* (Table 3 & Fig. 2). No NA1/34$^+$ cells of the epidermis were ever seen parasitized with mycobacteria.

The presence of RFD2$^+$ cells

Over 70% of non-lymphoid cells in the dermis of BL leprosy patients were RFD2$^+$ (Fig. 3 & Table 3). Indeed, RFD2$^+$ cells represented the majority of all mononuclear cells present. No consistent pattern of distribution was observed although the majority of these cells did tend to appear in clumps rather than being indescriminantly distributed throughout the infiltrate. ZN staining demonstrated that the majority of *M. leprae* present in the tissues, appeared within RFD2$^+$ cells (Fig. 3). No cells 'staining' with RFD2 were ever seen in the epidermis.

Overall distribution of mycobacteria

Combined immunohistology and ZN staining demonstrated that *M. leprae* could be found infecting all subpopulations of non lymphoid mononuclear cells in the dermal infiltrates (see above). The proportions of each cell type parasitized seemed approximately the same as the overall proportion of each cell type within the infiltrates (Table 3). This would imply that the mycobacteria were indiscriminate in the type of cell they infected.

Expression of HLA-DR antigens

Using indirect immunofluorescence methods combined staining with either RFD1, RFD2 or NA1/34 and RFDR2 was performed. These methods demonstrated that the majority of each of the cell subpopulations identified expressed HLA-DR antigens. However, the expression of HLA-DR was very variable and many of the cells showed either very weak staining or no staining at all. Using a combination of RFDR2 immunohistology and ZN staining it appeared that the parasitized cells expressed weaker HLA-DR. However, it was impossible to quantitate this observation and 'triple' staining (e.g. RFD1/RFDR2/ZN) proved technically unfeasible. One could not determine therefore which infected cell subpopulation failed to express HLA-DR. What was clearly revealed however was that in the epidermis the vast majority of epidermal Langerhans cells did not stain with RFDR2 (Fig. 4).

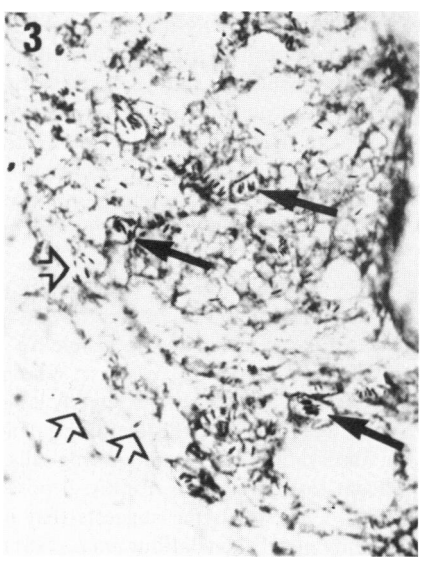


Fig. 3. Membrane staining of macrophages (RFD2$^+$) in the dermis of a patient with BL leprosy. Many of the cells contain *M. leprae* identified with a ZN stain. Three typical examples of parasitized cells are highlighted (closed arrows). Some *M. leprae* are clearly not within RFD2+ve cells (open arrows). Magnification × 400.

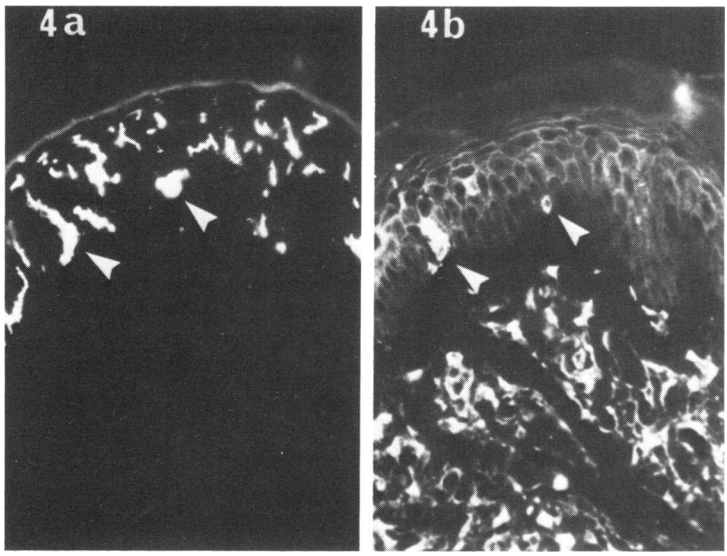


Fig. 4. Combined indirect immunofluorescence staining of a skin section from a patient with BL leprosy. The first layer reagents were NA1/34, (IgG) and RFDR2, (IgM). After washing a mixed second layer was applied containing an FITC conjugated goat anti-mouse IgG, and a TRITC conjugated goat anti-mouse IgM. (a) A photograph taken using selected filters for FITC, showing NA1/34$^+$ Langerhans cells in the epidermis. (b) A photograph taken of the same area using selective filters for TRITC. Although significant numbers of TRITC stained RFDR2$^+$ cells can be seen in the dermis; only two (arrowed) Langerhans cells are RFDR2$^+$. The vast majority of these cells are not expressing DR antigens. Magnification × 400.

DISCUSSION

This paper demonstrates that the majority of mononuclear cells in the dermal infiltrates of BL leprosy express the phenotype of macrophages. However, two other populations expressing phenotypic characteristics of interdigitating cells and Langerhans cells are also present. Of particular interest are the interdigitating cells. Originally described by Veldman in 1970, these interdigitating cells are thought responsible for antigen presentation to T lymphocytes in the interfollicular areas of lymph nodes (Balfour *et al.*, 1981). If, as has been suggested (Humphrey 1981) they are the human equivalent of the dendritic cells isolated from mice (Steinman & Cohn 1973), these cells are potent stimulators of T lymphocyte responses (Nussenszweigh *et al.*, 1980). Evidence in man to support this contention is that interdigitating cells express large amounts of HLA-DR antigens (Janossy *et al.*, 1981) a factor critical for the induction of T cell responses (Unanue, 1981).

Previous studies have demonstrated the presence of these cells in a variety of immunologically stimulated inflammatory reactions (for review see Poulter, 1983), where the presence of large numbers of these cells had been postulated as being responsible for T cell accumulation and the persistent and progressive nature of the inflammatory response. Whether or not interdigitating cells or indeed Langerhans cells are acting as APCs within the leprosy lesions remains unknown.

One key feature of leprosy however is that the aetiological agent is known and can be identified within the tissues. The observation that interdigitating cells and Langerhans cells can be infected with *M. leprae* is significant for two reasons. Firstly, it poses the question as to whether these cell types can be phagocytic. Current dogma suggests that interdigitating cells are non-phagocytic and Langerhans cells only poorly so (Balfour *et al.*, 1981). The observation that these cells can be infected with *M. leprae* could be taken to suggest that this is not the case. Alternatively, it could indicate that *M. leprae* can actively enter a cell, rather than passively enter via ingestion.

Secondly, the effects of infection on the functional capacity of antigen presenting cells, may be considerable. For example parasitism may inhibit antigen presenting capacity. The observations of reduced HLA-DR expression may indicate that functional capacity is impaired although this suggestion awaits more rigorous testing in experiments *in vitro*.

Although perhaps posing more questions than it answers the present study demonstrates a heterogeneity within the non-lymphoid cells of the BL associated inflammatory response and reveals that the infiltrates include cells with the phenotype of interdigitating cells and Langerhans cells. Furthermore, it suggests that these cell types, hitherto thought to be poorly or non-phagocytic, are, parasitized by the mycobacteria. The functional consequences of this parasitism are at present being investigated.

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