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Monoclonal antibodies distinguish macrophages and epithelioid cells in sarcoidosis and leprosy

C. S. MUNRO, D. A. CAMPBELL*, L. A. COLLINGS† & L. W. POULTER† *Host Defence Unit, Cardiothoracic Institute, Brompton Hospital, London SW3 6HP, and Departments of * Thoracic Medicine and † Immunology, Royal Free Hospital School of Medicine, London NW3 2QG, UK*

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SUMMARY

Existing anti-macrophage monoclonal antibodies are unable to differentiate between macrophages and epithelioid cells. In search of more precise reagents, we have applied recently developed antibodies to lesions of sarcoidosis and leprosy. UCHM1 and Leu-M3 stained both granulomas and surrounding histiocytes. However, in lesions with epithelioid granulomas there was a clear distinction between cells identified by RFD9 (epithelioid and giant cells) and RFD7 (macrophages in the surrounding mantle and normal tissue), whereas macrophages in the non-hypersensitivity granulomas of lepromatous leprosy were labelled by both the latter antibodies. In lung biopsies, alveolar macrophages were also labelled by both RFD7 and RFD9. These reagents may be useful for studying pathogenic mechanisms in granuloma formation.

Keywords sarcoidosis leprosy granulomas macrophages monoclonal antibodies

INTRODUCTION

Epithelioid cells (EC), such as occur in granulomas of sarcoidosis, tuberculosis and tuberculoid leprosy, are members of the mononuclear phagocyte series (MPS) (van Furth, 1982). Compared with resident tissue macrophages, EC exhibit increased lysosomal and respiratory enzyme activity (Williams & Jones Williams, 1983), but unlike activated macrophages, are poorly phagocytic and have reduced immunoglobulin and complement receptors (Turk & Narayanan, 1982). However, anti-macrophage antibodies used in immunohistological studies (OKM1: Bjerke, Matre & Nilsen, 1983; RFD2: Poulter *et al.*, 1984; FMC17 and PAM1: Hancock *et al.*, 1986) have so far failed to distinguish between EC and macrophages outside the granuloma. Since making such a distinction may facilitate the study of macrophage participation in granuloma formation, we have investigated recently developed reagents which identify subsets of mononuclear phagocytes for their reactivity in tissue involved by sarcoidosis and leprosy.

MATERIALS AND METHODS

Tissue specimens. Fifteen biopsies were taken from subjects with sarcoidosis (three lymph nodes, six skin lesions, including four mature positive Kveim responses, and six transbronchial biopsies);

Correspondence: Dr C. S. Munro, Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, UK.

all these biopsies contained well-formed epithelioid granulomas. Biopsies were also obtained from nine cases of leprosy. Of these, one had been classified as polar lepromatous (LL), three as borderline lepromatous (BL), three as borderline tuberculoid (BT), and two were positive Mitsuda reactions to lepromin tests in BT leprosy. Positive control tissues were surgical specimens of human palatine tonsil and normal skin, and uninvolved areas of lungs resected for carcinoma. Six-micrometre cryostat sections were studied with the following anti-macrophage monoclonal antibodies: Leu-M3 (anti-monocyte/macrophage, Becton-Dickinson; Dimitriu-Bona *et al.*, 1983); UCHM1 (anti-monocyte; Hogg *et al.*, 1984); RFD7 (anti-tissue macrophage; Poulter *et al.*, 1986) and RFD9 (antigerminal centre macrophage; Janossy *et al.*, 1986). Immunoperoxidase studies used peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako, High Wycombe, UK) and diaminobenzidine/hydrogen peroxide development. Combinations of the above reagents with RFDR1 (and IgM antibody to an HLR-DR framework epitope) were used in double immunofluorescence studies with class-specific fluorescein- and rhodamine-conjugated goat anti-mouse immunoglobulins (Southern Biotechnology Associates, Birmingham, Alabama, USA).

RESULTS

Sarcoidosis. In skin lesions, granulomas, perigranulomatous macrophages and tissue histiocytes were labelled by UCHM1 and Leu-M3 (Fig. 1a & b). However, RFD9 labelled membrane and cytoplasm of cells in granulomas only and RFD7 was negative in the granulomas but labelled dermal macrophages (Fig. 1c & d). Macrophages in perigranulomatous mononuclear cell infiltrates were also RFD7 positive. HLA-DR bearing cell were found among both $RFD9^+$ and $RFD7^+$ populations. The same pattern was seen in the lymph nodes. The expression of UCHM1, Leu-M3 and RFD9 markers tended to decay towards the centre of large granulomas (Fig. 2a). RFD9 was strongly positive on giant cells (Fig. 2b). Again, there was no expression of RFD7 within granulomas, but the marker was present on sinus histiocytes, on which it is found in normal nodes. In pulmonary lesions there was also a clear distinction between RFD9 positive epithelioid cells and RFD7 positive macrophages found in perigranulomatous tissue (Fig. 2c & d). However, subpopulations of alveolar macrophages, which were virtually all HLA-DR positive, were labelled by all four antibodies. In one case, the granuloma was weakly labelled by RFD7; this was a biopsy from chronic pulmonary sarcoidosis in which T cells within the granuloma were notably rare.

Leprosy. UCHM1 and Leu-M3 again labelled granulomas and surrounding cells in all lesions, although Leu-M3 positive cells were fewer in the centre of lesions. However, those containing distinct epithelioid granulomas (BT lesions and positive Mitsuda responses) were labelled by RFD7 and RFD9 in the same pattern as cutaneous sarcoid lesions (Fig. 3a & b). In the BL and LL lesions, macrophages in the infiltrates expressed both RFD7 and RFD9 markers (Fig. 3c & d), although in the BL lesions there were areas of poorly formed granulomas in which macrophages were RFD7 negative.

DISCUSSION

The distinctions made possible by RFD9 and RFD7 are useful, since UCHM1, Leu-M3, and other reagents identifying macrophage and monocyte populations and subpopulations which we have tried have not proved of practical value in discriminating EC and macrophages in cryostat sections. Neither RFD7 or RFD9 markers are found on blood monocytes but are present on macrophages in other circumstances (Poulter *et al.* 1986; Janossy *et al.* 1986); hence their expression is acquired during differentiation. RFD9 is not a specific marker of macrophages in granulomas, since it also labels those in lymph node germinal centres. These regions have in common the presence of helper ($T4^+$) lymphocytes. Moreover, like HLA-DR, expression of the marker is strong on giant cells. However, the development of the antigen recognized by RFD9 is a feature of macrophage differentiation distinct from increased HLA-DR expression, since $RFD7^+$ cells around granulomas also bear HLA-DR, and $RFD9^+$ cells are found in lepromatous infiltrates, in which HLA-DR expression is weak (Collings, Waters & Poulter 1985).

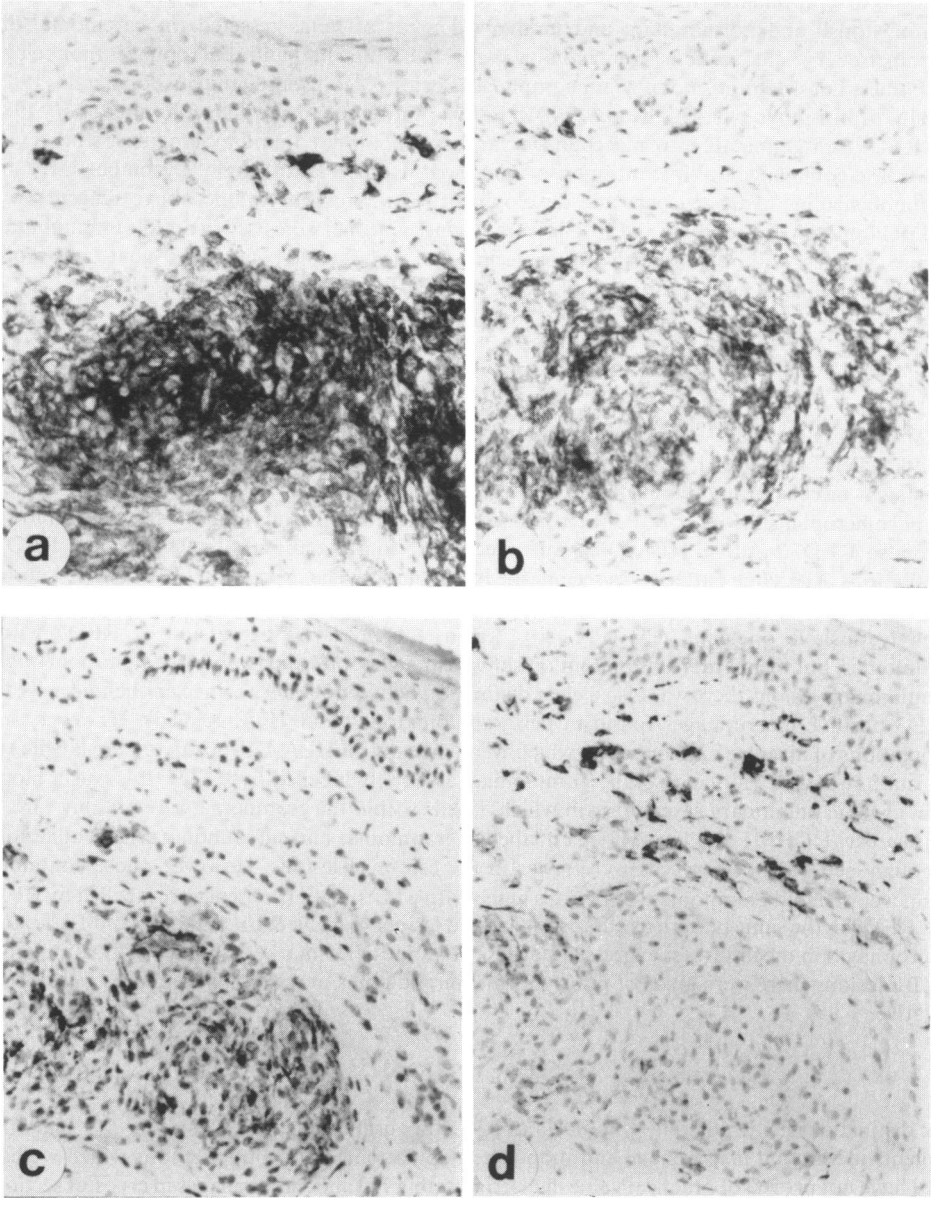

Fig. 1. A cutaneous sarcoid lesion. Immunoperoxidase; haematoxylin counterstain (× 200). (a) UCHM1 and (b) Leu-M3. Cells within and around the granuloma are identified by both antibodies; (c) RFD9: cytoplasmic and some membrane staining within the granuloma only; (d) RFD7: the granuloma is negative but dermal macrophages are positive.

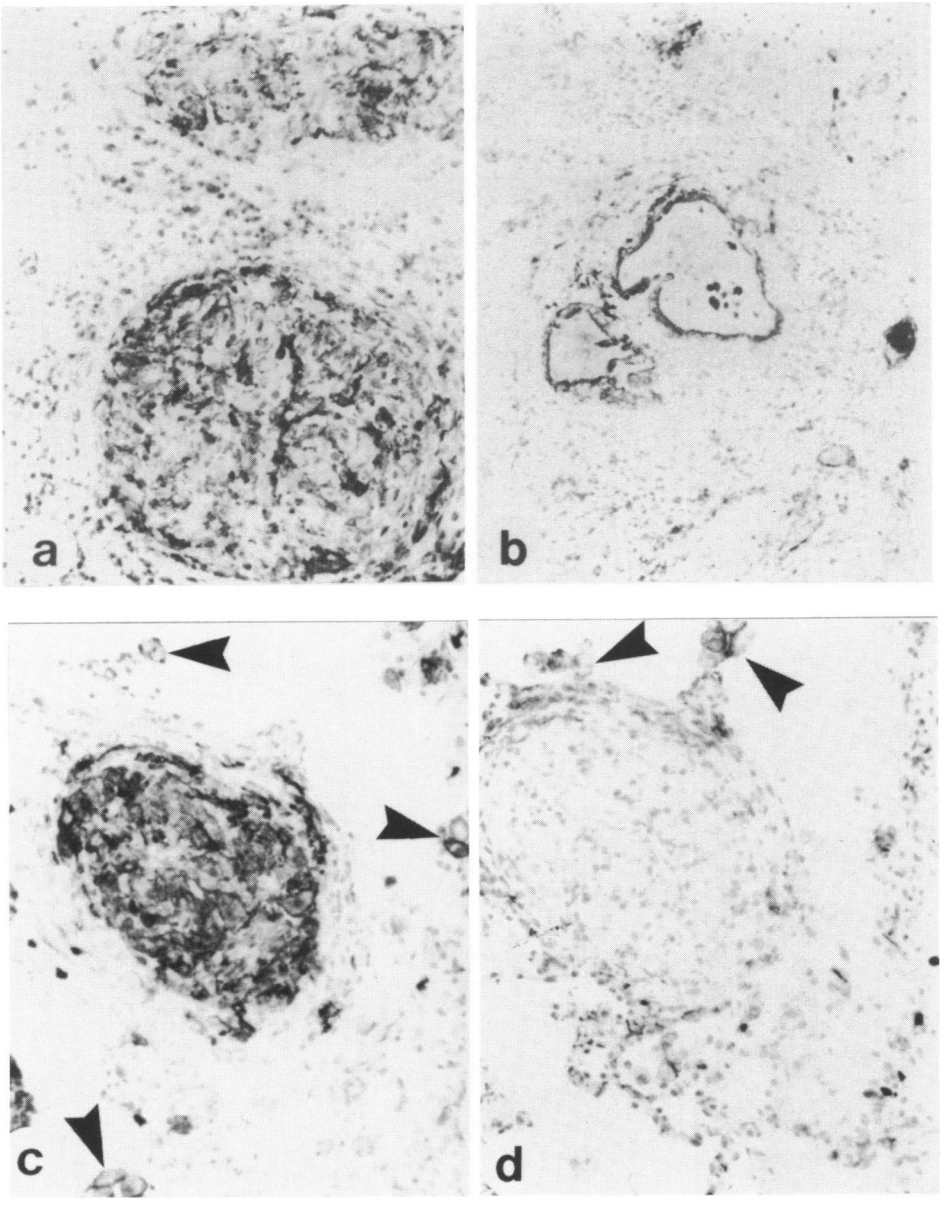

Fig. 2. Lesions of sarcoidosis. Immunoperoxidase; haematoxylin counterstain (× 200). (a) RFD9 on a lymph node: labelling tends to be less in the centre of granulomas. (b) the same: strongly positive giant cells. (c) RFD9 and (d) RFD7 on adjacent sections of a transbronchial biopsy. Despite distinguishing epithelioid cells and tissue macrophages, both reagents label some alveolar macrophages (arrowed).

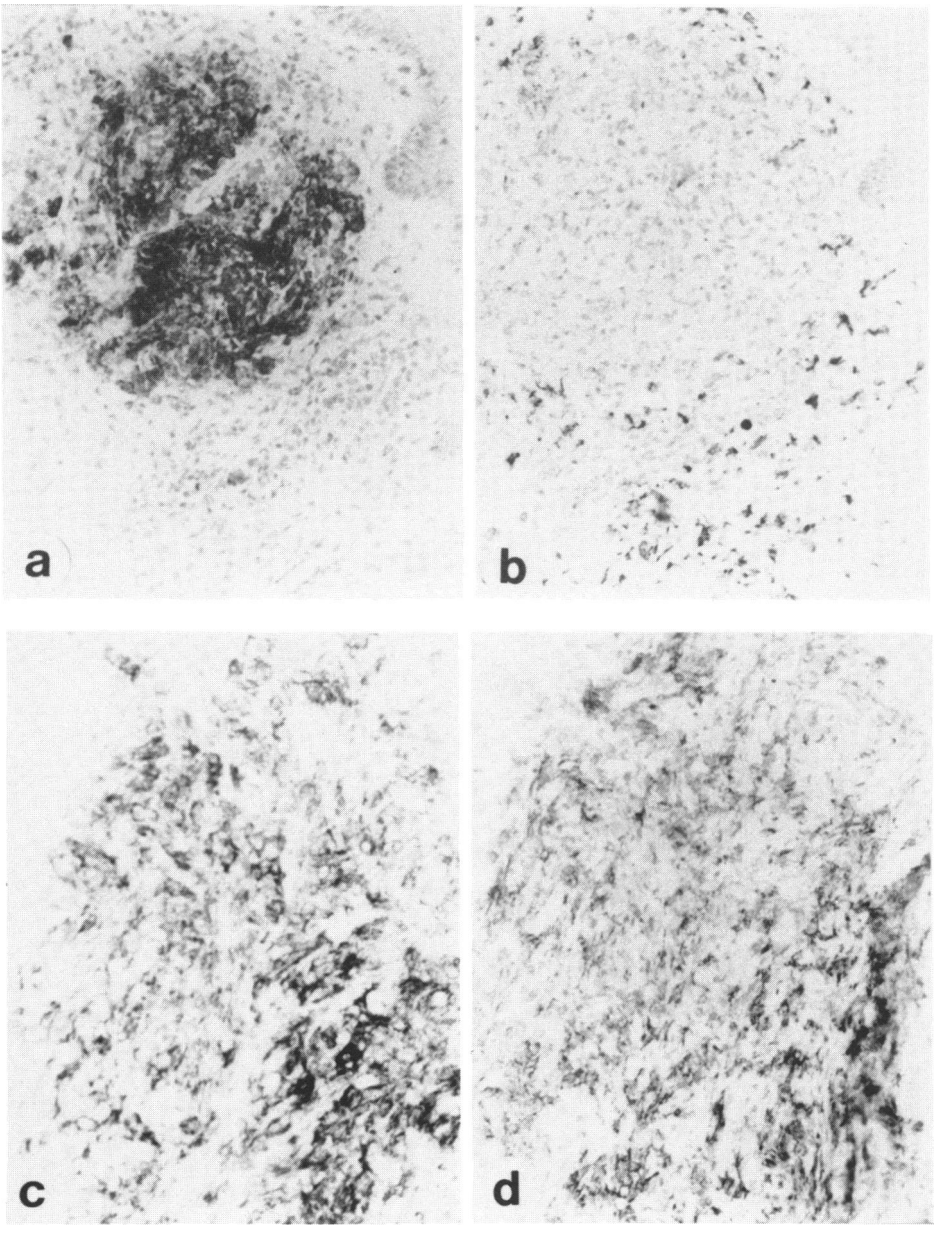

Fig. 3. Lesions of leprosy. Immunoperoxidase; haematoxylin counterstain (× 200). (a) RFD9 and (b) RFD7 on adjacent sections of Mitsuda reaction to a lepromin test. The reciprocal labelling is again seen. (c) RFD9 and (d) RFD7 on adjacent sections of a lepromatous infiltrate. Both reagents label macrophages in the infiltrate.

The absence of the RFD7 marker is more characteristic of EC than the presence of that of RFD9. Since tissue macrophages are RFD7$^+$ (Poulter *et al.*, 1986), the fact that epithelioid cells are RFD7$^-$ may parallel their loss of phagocytic capacity and immunoglobulin and complement receptors (Turk & Narayanan, 1982; Williams & Jones Williams, 1983). The RFD7 marker develops during maturation of human peripheral blood monocytes *in vitro*, but its appearance is prevented by gamma-interferon (L. W. Poulter, G. A. W. Rook, J. Steel & A. Condez, unpublished). This mediator has been identified in sarcoid granulomas (Hancock *et al.*, 1986), and hence the phenotype of epithelioid cells may in part be due to lymphokines released by activated T cells, consistent with the well-documented importance of cell-mediated immunity in epithelioid granuloma formation (Boros, 1983). This suggestion is reinforced by the finding of RFD7 positive macrophages throughout the infiltrates of lepromatous leprosy, since the latter form of disease is associated with poor cell-mediated immune response. It is of interest that while RFD7 does not label epithelioid granulomas, macrophages bearing the marker are found immediately around them, implying that any microenvironmental factors regulating phenotype are highly localized.

Together, these results define a phenotype associated with cells of the MPS in immunologically-mediated (RFD9$^+$/RFD7$^-$ and HLA-DR$^+$) as opposed to those of non-immunologically-mediated (RFD9$^+$/RFD7$^+$ and HLA-DR$^{+/-}$) granulomas. Further investigation of the antigens identified by these reagents, the functional capacity of macrophages which express them, and the factors which influence their expression may help to elucidate pathogenic processes in granulomatous disorders.

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