Clin. exp. Immunol. (1990) **81**, 123–126

Differential expression of the calcium-binding proteins MRP8 and MRP14 in granulomatous conditions: an immunohistochemical study

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(Accepted for publication 8 March 1990)

SUMMARY

MRP14 and MRP8 are well-characterized calcium-binding proteins present in myeloid cells and mononuclear phagocytes. These antigens can easily be visualized in paraffin-embedded tissue, making use of monospecific polyclonal antibodies. This study evaluates MRP14 and MRP8 expression in mononuclear phagocytes in various granulomatous conditions. MRP14 is strongly expressed in all granulomatous conditions. MRP8 is variably expressed. Mononuclear phagocytes in granulomas of foreign body type, cat-scratch disease and erythema nodosum strongly express MRP8. In contrast, MRP8 expression is weak or absent in mononuclear phagocytes of sarcoidosis and tuberculosis. These results show differences in immunophenotype between non-phagocytic mononuclear phagocytes in delayed hypersensitivity type granulomas and phagocytic mononuclear phagocytes in non-hypersensitivity and non-immunological granulomas.

Keywords granuloma immunohistochemistry delayed hypersensitivity

INTRODUCTION

Granulomas can be divided into immunological types and non-immunological types (Warren, 1976). The immunological types comprise cell-mediated and immune complex-mechanisms of inflammation. Sarcoidosis and tuberculosis are examples of cell-mediated delayed hypersensitivity type granulomas (Semenzato, 1988; Kaufmann & Flesch, 1988), where interactions between T cells and mononuclear phagocytes play an important role. Immune complexes play an important role in granulomatous inflammation such as extrinsic allergic alveolitis (Seal, Edwards & Hayes, 1975), and probably also in erythema nodosum (Niemi *et al.*, 1977). The persistence of immune complexes causes mononuclear phagocyte recruitment (Spector & Heesom, 1969). Most foreign body granulomas represent non-immunological granulomas. Mononuclear phagocytes in these granulomas are recruited by a variety of chemical substances (Epstein, 1980).

Few immunohistochemical markers can differentiate mononuclear phagocytes involved in different types of granulomatous inflammation, except for HLA-DR expression which is increased in mononuclear phagocytes of delayed hypersensitivity type granulomas (Van den Oord *et al.*, 1984). Other markers have been described by Munro *et al.* (1987), who reported on two monoclonal antibodies, RFD7 and RFD9, which can differentiate mononuclear phagocytes in the mantle of epitheliod granulomas and epitheloid cells composing the granulomas. We studied a series of granulomatous conditions with recently developed polyclonal antibodies identifying MRP8 and MRP14. MRP8 and MRP14 are cytosolic calcium-binding proteins (MW 8 kD and 14 kD, respectively) present in granulocytes, monocytes and a subset of histiocytes (Odink *et al.*, 1987). MRP8 and MRP14 are part of a complex, known as the L1 antigen (Dale *et al.*, 1985; Brandtzaeg, Dale & Fagerhol, 1987). This complex can also be recognized by the monoclonal antibody Mac387 (Flavell, Jones & Wright, 1987).

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MATERIALS AND METHODS

The material used and the histological features of the biopsies are listed in Table 1.

All biopsies were fixed in B5 and routinely processed to paraffin. Production and characterization of the monospecific polyclonal antibodies directed against MRP8 and MRP14 have been described elsewhere (Odink *et al.*, 1987). Monoclonal antibody KP1 was kindly provided by Dr D. Y. Mason (Nuffield Department of Pathology, Oxford, UK). KP1 recognizes a heavily glycosylated molecule (MW 110 kD) present in myeloid and mononuclear phagocyte cells (Pulford *et al.*, 1989). Monoclonal anti-HLA-DR antibody TAL1B5 was kindly provided by Dr W. F. Bodmer (Imperial Cancer Research Fund, London, UK) (Adams, Bodmer & Bodmer, 1983). Anti-MRP8 and anti-MRP14 were diluted 1/2000 and 1/4000, respectively.

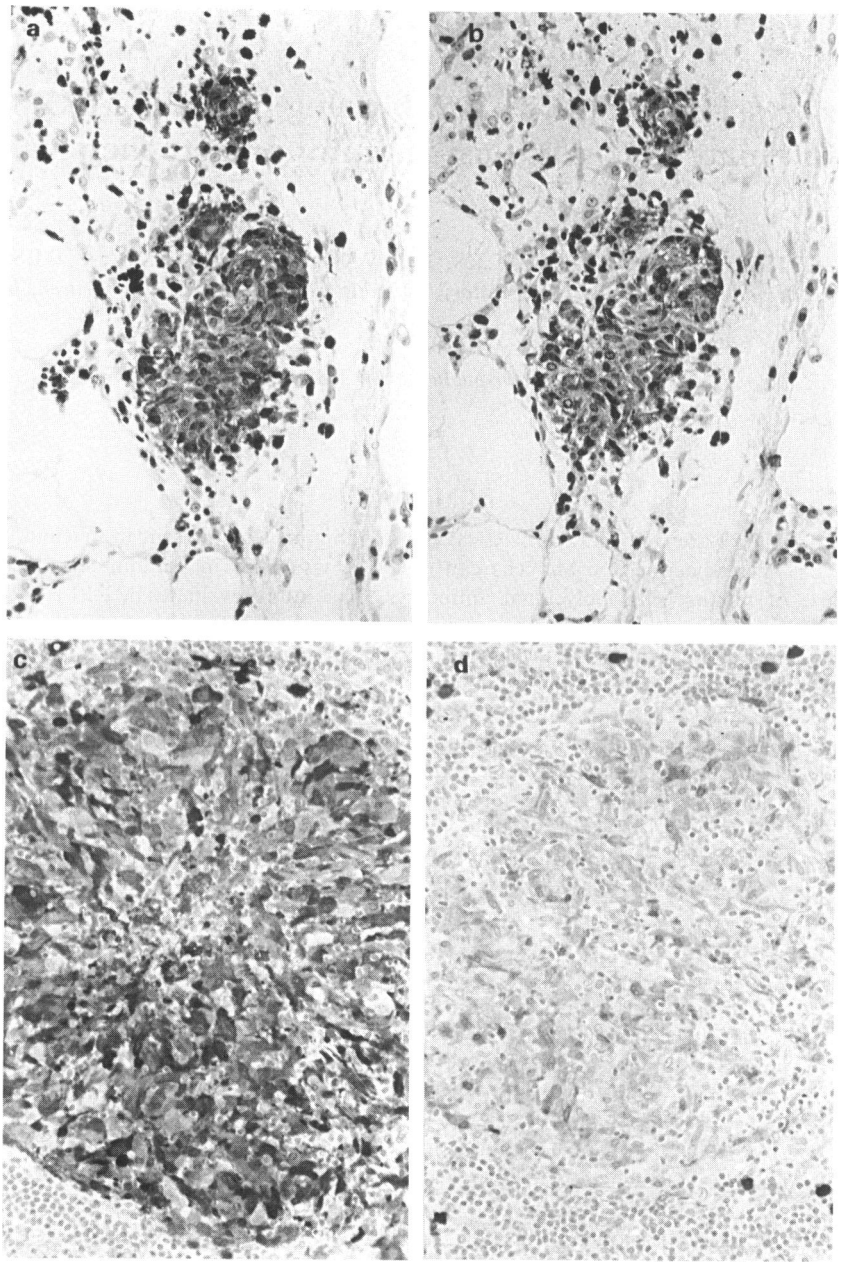

Fig. 1. Semi-serial sections of a granuloma in a skin biopsy showing the histological features of erythema nodosum, stained for MRP14 (a) and MRP8 (b). Both MRP14 and MRP8 are expressed by mononuclear phagocytes composing the granuloma.

Semi-serial sections of a tuberculous granuloma in a lymph node, stained for MRP14 (c) and MRP8 (d). Only MRP14 is strongly expressed, while MRP8 expression is weak or absent in most mononuclear phagocytes composing the granuloma. MRP8 expression however is pronounced in some mononuclear phagocytes surrounding the granuloma. Magnification × 140.

They were used in a three-step, unlabelled antibody enzyme method using the peroxidase–anti-peroxidase complex (Dakopatts, Copenhagen, Denmark) as described previously (Hsu, Raine & Fanger, 1981). KP1 and TAL1B5 were diluted 1/10 and 1/5, respectively. They were used in a three-stage avidin–biotin complex (Dakopatts) method as described previously (Hsu *et al.*, 1981). The peroxidase reaction product was developed using a solution of diaminobenzidine–H_2O_2 and the sections were then counterstained with Mayer's haematoxylin.

RESULTS

Small histiocytes, epitheloid cells and multi-nucleated giant cells, which are all subtypes of mononuclear phagocytes, reacted strongly MRP8 and MRP14 in foreign body granulomas, erythema nodosum and cat-scratch disease (Fig. 1). Small histiocytes, epitheloid cells and multi-nucleated giant cells also stained with KP1. KP1 staining was granular, and dispersed throughout the cytoplasm. HLA-DR expression was generally

Table 1. Material used in this study

Tissue	Diagnosis	*n*	Histology
Skin	Foreign body type granuloma (ruptured epidermal cyst)	3	Diffuse granulomatous infiltrate. Epitheloid cells and multi-nucleated giant cells surrounding squames and cholesterol clefts; few neutrophils
	Erythema nodosum	3	Small epitheloid granulomas present in the subcutaneous fat septa
	Sarcoidosis	2	Well-delineated granulomas composed of epitheloid cells, lymphocytes and multi-nucleated giant cells
	Tuberculosis	3	Variably sized granulomas composed of epitheloid cells, lymphocytes and multi-nucleated giant cells; occasionally central caseous necrosis
Lymph node	Cat-scratch disease	3	Variably sized granulomas forming palissading rings of epitheloid cells surrounding collections of neutrophils; few lymphocytes
	Sarcoidosis	3	Well-delineated granulomas composed of epitheloid cells, lymphocytes and multi-nucleated giant cells
	Tuberculosis	4	Variably sized granulomas composed of epitheloid cells, lymphocytes and multi-nucleated giant cells; occasionally central caseous necrosis

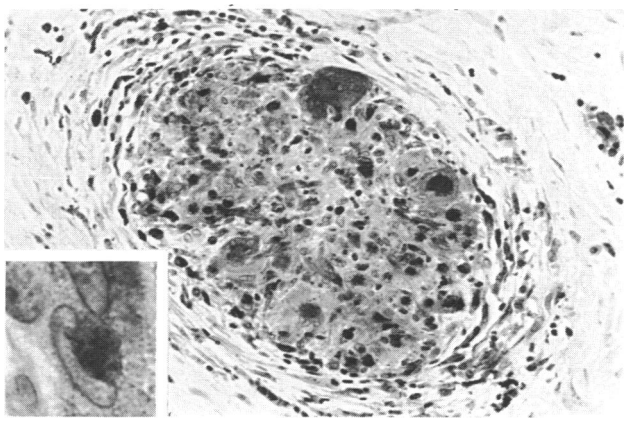

Fig. 2. Sarcoidal granuloma in a skin biopsy stained with KP1. KP1 staining is dot-like in most mononuclear cells, including epitheloid cells (inset) and multi-nucleated giant cells. An occasional giant cell is diffusely stained with KP1. Magnification × 140; inset × 550.

weak in these cells. Epitheliod cells and multi-nucleated giant cells composing granulomas in sarcoidosis and tuberculosis expressed MRP14 strongly. MRP8 expression in these cells was weak or absent (Fig. 1). However, in the latter conditions, small histiocytes surrounding the granulomas expressed strongly both MRP14 and MRP8. Epitheloid cells and multi-nucleated giant cells in tuberculosis and sarcoidosis stained with KP1 and for HLA-DR. KP1 staining was granular, but frequently dot-like and sometimes restricted to the paranuclear region (Fig. 2). HLA-DR expression was pronounced, although variable.

DISCUSSION

While most mononuclear phagocyte markers do not allow a distinction between mononuclear phagocytes involved in delayed hypersensitivity type granulomas (e.g. sarcoidosis and tuberculosis) and those involved in non-hypersensitivity and non-immunological granulomas, these cells were stained differently for MRP14 and MRP8.

MRP14 and MRP8 are both strongly expressed in all mononuclear phagocytes of erythema nodosum, cat-scratch disease and foreign body type granulomas. Mononuclear phagocytes in these non-hypersensitivity and non-immunological granulomas probably exert a phagocyting function. The involvement of an Arthus-type reaction with deposition of immune complexes (Niemi *et al.*, 1977) or the presence of Gram-negative bacteria (Wear *et al.*, 1983) or foreign material in these conditions provide circumstantial evidence for this speculation. In contrast, MRP14 is expressed strongly in mononuclear phagocytes in sarcoidosis and tuberculosis, while MRP8 is expressed weakly or even absent in most of these cells. Sarcoidosis and tuberculosis represent T cell-mediated immune responses (Semenzato, 1988; Kaufmann & Flesch, 1988).

A similar distinction between mononuclear phagocytes involved in hypersensitivity type and non-hypersensitivity granulomas was noted previously by Munro *et al.*, (1987) using monoclonal antibodies RFD7 and RFD9 on frozen tissues. RFD7 and RFD9 identified mononuclear phagocytes in non-hypersensitivity granulomas of lepromatous lepra. In sarcoid granulomas and borderline tuberculoid lepra epitheloid cells, which are the main subtype of mononuclear phagocytes in these conditions, stained only for RFD9. RFD7 positivity is correlated with phagocytic capacity while loss of RFD7 staining reflects loss in phagocytic capacity. Despite comparable findings and similar conclusions drawn from them, the antigens recognized by RFD7, RFD9 on the one hand and the MRP8, MRP14 antigens on the other hand are probably not related. MRP8 and MRP14 have molecular weights of 8 kD and 14 kD, respectively, and are present in circulating monocytes (Odink *et al.*, 1987). RFD7 and RFD9 recognize molecules with a different molecular weight, which are not present in circulating monocytes, but which are acquired during differentiation (Poulter *et al.*, 1986; Munro *et al.*, 1987).

This study shows differences in MRP14 and MRP8 expression in mononuclear phagocytes in hypersensitivity type granulomas and non-immunological and non-hypersensitivity granulomas. Both antigens are well characterized. Their functions, however, are not yet understood.

ACKNOWLEDGMENTS

Thanks are due to Dr K. Odink (Ciba/Geigy, Basel) for providing the polyclonal antibodies recognizing MRP8 and MRP14, to Dr D. Y.

Mason for providing monoclonal antibody KP1, and to Dr W. F. Bodmer for providing monoclonal antibody TAL1B5. We thank B. Smets, E. Vandessel for excellent technical assistance, M. Rooseleers for preparing the photographs and M. Goedhuys for typing the manuscript.

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