The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80

J. Cell Sci. **66**, 189–194 (1984)
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THE MONONUCLEAR PHAGOCYTE SYSTEM OF THE MOUSE DEFINED BY IMMUNOHISTOCHEMICAL LOCALIZATION OF ANTIGEN F4/80: MACROPHAGES OF BONE AND ASSOCIATED CONNECTIVE TISSUE

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SUMMARY

The macrophage-specific antigen F4/80 has been localized in adult mouse bone and connective tissue. F4/80 positive cells form the centre of haemopoietic islands, line the periosteal and subendosteal bone surfaces and are a major component of connective tissue and the synovial membrane (presumptive type A cells). F4/80 is absent from fibroblasts, chondrocytes, osteoblasts, osteocytes, osteoclasts and a subpopulation of synovial lining cells (presumptive type B cells).

INTRODUCTION

F4/80 is a rat hybridoma secreting a monoclonal antibody directed against the 160×10^3 molecular weight plasma membrane component of mouse mononuclear phagocytes (Austyn & Gordon, 1981). Previous reports have provided evidence that F4/80 antigen is a differentiation marker for macrophages (Hirsch, Austyn & Gordon, 1981) and is restricted to this cell lineage in major lymphoid and non-lymphoid tissues (Hume & Gordon, 1983; Hume, Robinson, MacPherson & Gordon, 1983*a*; Hume, Perry & Gordon, 1983*b*). The present paper is concerned with the immunohistochemical localization of the antigen in mouse bone and associated-connective tissue and investigates the origins of synovial lining cells and osteoclasts.

MATERIALS AND METHODS

Adult male Balb/c mice were perfused through their aortas with 0·5% (v/v) glutaraldehyde in 1% (w/v) sucrose/0·1 M-cacodylate as described by Hume & Gordon (1983). The femur and knee joint were excised and decalcified by incubation (24–48 h) in several changes of acid/citrate buffer (13% (w/v) sodium citrate in 2% (v/v) formaldehyde; pH 4·7 with formic acid). Tissue was embedded in Polywax (Difco, U.K.) and 6 μm sections were cut and stained for F4/80 antigen by

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the indirect ABC immunoperoxidase technique of Hsu, Raine & Fanger (1981) as modified by Hume & Gordon (1983). Reagents were supplied by Vector Laboratories (Kit PK 4004) through Sera-Lab, U.K. Control sections with irrelevant rat monoclonal antibodies, or without first antibody, were devoid of reaction product. Sections were counterstained with Mayer's haematoxylin and photographed with a blue (Ilford 303) filter to increase contrast.

RESULTS AND DISCUSSION

Previous immunohistochemical studies using F4/80 have led to the proposal that the antigen is restricted to mononuclear phagocytes and is absent from all other well-defined cell types (Hume & Gordon, 1983; Hume *et al.* 1983*a,b*). The present study adds to the latter category in that chondrocytes, fibroblasts, osteocytes, bone-lining cells, osteoblasts and osteoclasts are unstained by immunohistochemical localization of F4/80 (see below). Although these cells are unstained, bone and adjacent connective tissue contain an abundant population of $F4/80^+$ cells. $F4/80^+$ cells share the common features of stellate morphology (apart from occasional cells resembling monocytes) and a tendency to spread on surfaces or to intercalate with other cells. As noted elsewhere (Hume *et al.* 1983*a*), the most prominent population of $F4/80^+$ cells is found in the marrow forming the centre of haemopoietic islands. The distribution of these cells appears uniform throughout the marrow and there is no evidence of differential association with erythropoietic *versus* granulopoietic islands. Examples of such cells can be seen in Fig. 1.

The surfaces of the bone are coated with a narrow layer of bone-lining cells, which may be quiescent osteoblasts (Jee, 1983). Immediately adjacent to the bone lining, on both the subendosteal and periosteal faces, is an extensive layer of $F4/80^+$ cells spread in the plane of the surface. These cells are difficult to see in vertical sections because

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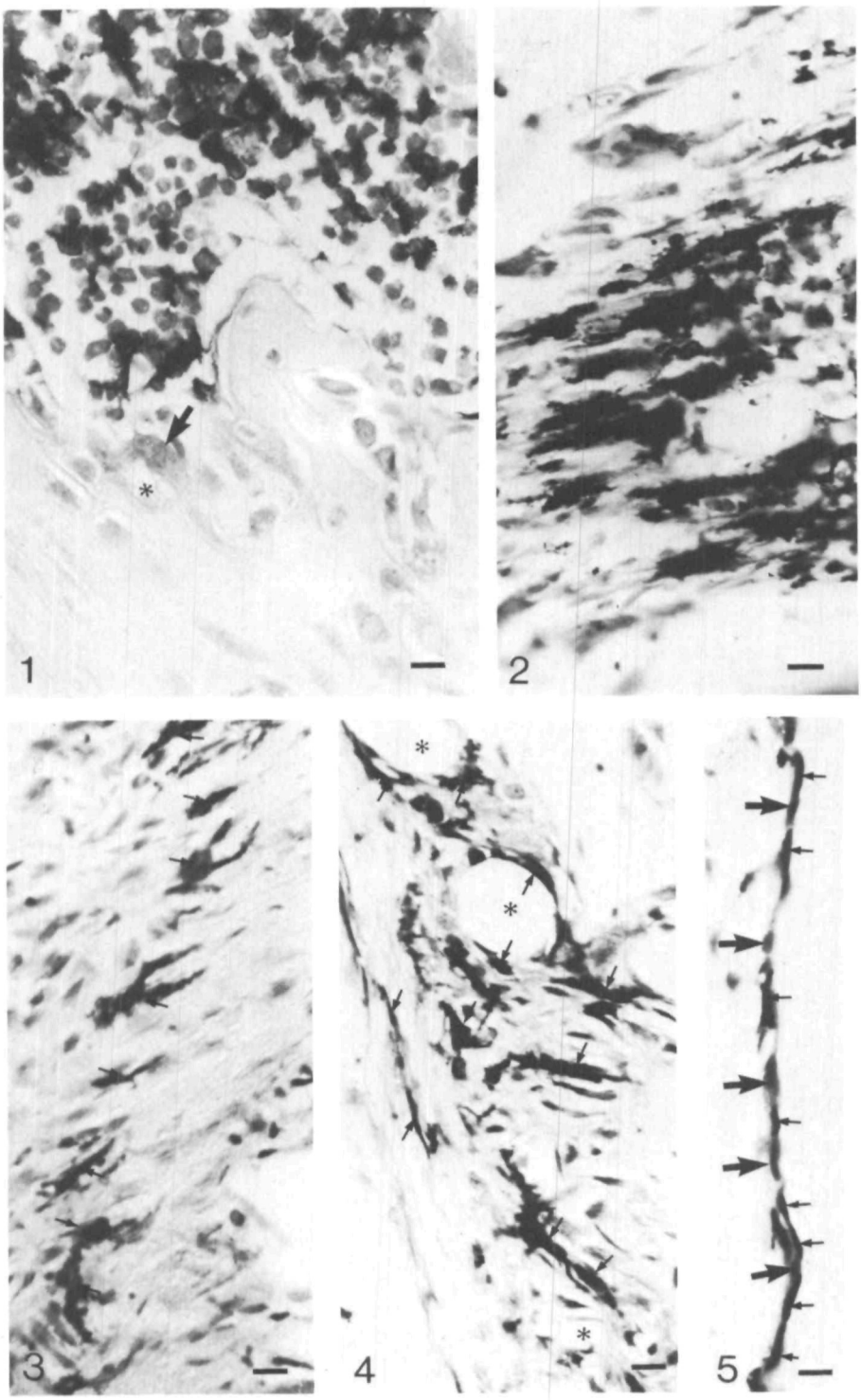
Fig. 1. Metaphyseal growth plate: numerous F4/80 positive cells (dark precipitate) can be seen in the marrow in the upper half of the field. Note the stellate morphology of the $F4/80^+$ cells and the apparent attachment of numerous smaller developing haemopoietic cells. $F4/80^+$ cells cannot be seen immediately adjacent to the bone surface. Large arrow indicates a multinucleate osteoclast that is unstained with F4/80. The ruffled border of the cell faces an indentation in the bone surface (asterisk indicates lacuna). F4/80 is also absent from bone-forming cells in the lower half of the field. Bar, 25 μm.

Fig. 2. An oblique section of the subendosteal bone surface showing an almost continuous layer of $F4/80^+$ cells (dark precipitate) spread in the plane of the bone. Bar, 10 μm.

Fig. 3. An oblique section of the periosteal bone surface. The angle of section is greater than in Fig. 2 so that the periosteal $F4/80^+$ cell lining appears as a line of stained stellate cells (arrows). These cells lie outside the bone-lining cells (left of field) and inside a layer of fibrous connective tissue (right of field). Bar, 10 μm.

Fig. 4. Highly vascularized (asterisks indicate vessels) loose connective tissue of the synovial lining contains many $F4/80^+$ cells (arrows) frequently associated with the blood vessels. The left of the field contains a more ordered array of fibrous connective tissue with $F4/80^+$ cells flattened like fibroblasts between the fibres. The field also contains many F4/80 negative cells, most of which are fibroblasts. Bar, 10 μm.

Fig. 5. Synovial membrane: $F4/80^+$ (small arrows) and $F4/80^-$ cells (large arrows) are present in roughly equal numbers. The contrast is difficult to demonstrate unequivocally in black and white micrographs but is obvious in the original colours. Bar, 10 μm.



Figs 1–5

of their thin cytoplasm. The density of the covering can be assessed from Figs 2 and 3, where oblique sections have grazed the subendosteal and periosteal surfaces, respectively. The tendency of F4/80$^+$ cells to associate with bone surfaces is also seen on bone spicules within the marrow (not shown) but is less evident than in the vicinity of the growth plate (Fig. 1).

Towards the joint the periosteum becomes lined with connective tissue. In dense fibrous connective tissue the F4/80$^+$ macrophages, like the unlabelled fibroblasts that are present in roughly equal numbers, lie flattened between the ordered collagen fibres (see Fig. 6). In loose, well-vascularized connective tissue including that underlying the synovial membrane (Fig. 4) F4/80$^+$ cells are equally numerous and often have cell bodies immediately adjacent to the blood vessels (pericytes) as noted in many other tissues (Hume *et al.* unpublished). The synovial membrane itself contains an F4/80$^+$ population. Approximately 40–50 % of the synovial lining cells express the antigen. The cells extend membrane processes between the unlabelled cells so that the staining appears almost continuous (Fig. 5). The identification of F4/80 on a subpopulation of synovial lining cells is in agreement with its restriction to mononuclear phagocytes, since synovial type A cells have morphological and functional features of macrophages and are of bone marrow origin (Edwards, 1982). The presence of an F4/80 negative population of synovial cells (presumed to be the type B cells) argues

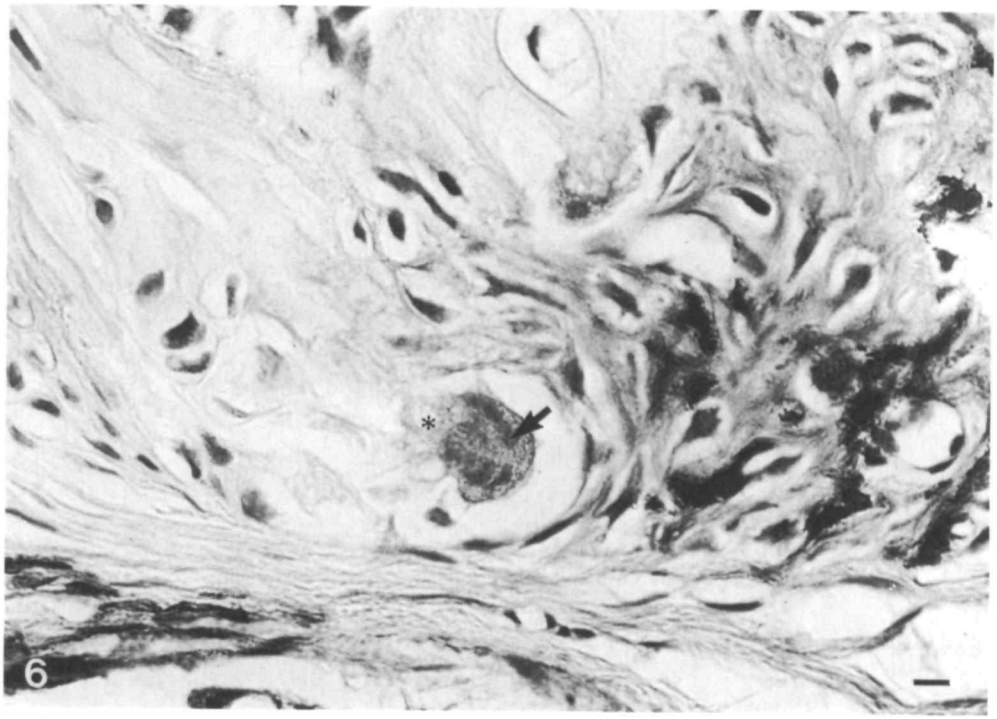


Fig. 6. High-power view of F4/80 negative multinucleate osteoclast (arrow) with ruffled border (asterisk). F4/80$^+$ cells (dark precipitate) can be seen in dense disordered connective tissue on the right and flattened between collagen fibres on the lower side of the field. Bar, 6 μm.

against the concept expressed by Ghadially (1978) that 'type A and type B cells are not distinct and different races of cells with distinct and different functions, but merely cells whose morphology reflects the function they are performing at a given moment'. Type B cells have the morphological appearance of fibroblasts (Jee, 1983) and this would be their most obvious source.

The absence of F4/80 from osteoclasts is of particular importance because of continued debate over the origin of these cells (Loutit & Nisbet, 1982). Figs 1 and 6 contain F4/80 negative osteoclasts identifiable by their morphology (including ruffled border), multinuclearity and location adjacent to a lacuna in the bone surface. An examination of over 40 such cells failed to identify any staining with F4/80. Some authors favour the view that osteoclasts are polykaryons formed by the fusion of mature mononuclear phagocytes (see Loutit & Nisbet, 1982; Ericsson, 1980). Support for this view can be taken from the observed ability of isolated macrophage populations to degrade bone matrix (Mundy, Altman, Gondek & Bandellin, 1977; Rifkin, Baker & Coleman, 1979; Holtrop, Cox & Glowacki, 1982) but macrophages resorbing bone do not form osteoclasts (Rifkin *et al.* 1979; Burger *et al.* 1982). Burger *et al.* (1982) found that the osteoclast progenitor was contained in the weakly adherent fraction of proliferating bone marrow cultures composed primarily of macrophages. They concluded that the most likely precursor was the monoblast or promonocyte. F4/80 antigen is present on immature, non-adherent, macrophage progenitors in marrow cultures (Hirsch *et al.* 1981) and on immature macrophages in bone marrow *in vivo* (Hume *et al.* 1983*a*). Thus the pathway suggested by Burger *et al.* (1982) would probably involve loss of F4/80 antigen during osteoclast formation. Alternatively, the results of Burger *et al.* (1982) do not eliminate the possibility that bone marrow cultures contain a specialized osteoclast 'stem cell' (Loutit & Nisbet, 1982) and that the absence of F4/80 from osteoclasts is a reflection of its restriction to mononuclear phagocytes.

This work was supported in part by a grant from the Medical Research Council, U.K. We thank N. W. Nisbet for advice and materials; Mr Don Etheridge for assistance with histology, Mr Stan Buckingham and Miss Gill Roberts for photography and Mrs Pam Woodward for typing the manuscript.

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(Received 1 September 1983–Accepted 12 September 1983)