Brief Definitive Report

MONONUCLEAR PHAGOCYTE SYSTEM OF THE MOUSE DEFINED BY IMMUNOHISTOCHEMICAL LOCALIZATION OF ANTIGEN F4/80

Identification of Resident Macrophages in Renal Medullary and Cortical Interstitium and the Juxtaglomerular Complex*

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F4/80 is a hybridoma that secretes a noncytotoxic rat IgG2b directed against a protein determinant on mouse macrophage plasma membranes (1). The antigen is contained in a 160,000 mol wt glycoprotein. Unlike other markers present on mouse macrophages (2, 3), F4/80 is not expressed on any other blood cell type (1). Furthermore, it is absent from the Steinman-Cohn dendritic cell found in mouse lymphoid tissues (4). During differentiation F4/80 appears first on preadherent macrophage progenitors and increases with maturation. It is present on all independent macrophage colonies derived in vitro (5).

The F4/80 antigen is extremely stable. This observation has allowed us to develop a technique for immunohistochemical localization of the antigen in tissue sections, using adequate fixation to maintain tissue morphology. Examination of many mouse tissues has confirmed that antigen F4/80 is present on all well-defined macrophage populations and is not present on any cell type other than a macrophage (manuscript in preparation). Of relevance to the present report is the absence of F4/80 from endothelial and epithelial cells and fibroblasts.

The kidney is not commonly regarded as an organ that contains large numbers of macrophages in the absence of inflammation. However, resident macrophages in the peritoneum and lung possess two biochemical activities that are particularly relevant to the kidney: a unique ability to produce arachidonic acid metabolites (prostaglandins E, $F_{2\alpha}$, prostacyclin, thromboxanes, hydroxy-eicosatetraenoic acids, and leukotrienes) (6–9; see reference 10 for a review) and a steroid-inducible angiotensin I-converting enzyme (11, 12). There has been considerable interest in the interplay between bradykinin, angiotensin II, and prostaglandins in the regulation of glomerular ultrafiltration and renal fluid and salt retention (13-18). The cells producing these regulators have not been adequately identified. We report here that macrophages are present throughout the kidney and are particularly concentrated amongst the medullary interstitial cells and in the vicinity of glomeruli.

Materials and Methods

F4/80 is not restricted to any mouse strain. The illustrations are from studies of CBA/T6T6 male mice, but outbred and other inbred strains give similar results. The method of immunoperoxidase staining is based on that of Hsu et al. (19) with reagents (Vectastain Kit, PK 4004) supplied by Sera-Lab Ltd., Crawley Down, West Sussex, England. Adult mice were anaesthetized with ether and perfused by inserting a plastic cannula (pp50) through the left ventricle and tying into the aorta. The inferior vena cava was cut below the liver (above the renal veins) and the blood was cleared by perfusion with heparinized saline for 2–3 min. The animals were then perfused with fixative (0.5% glutaraldehyde [E. M. grade; Polysciences, U.K. Ltd., UK] in 0.1 M cacodylate buffer, pH 7.4, plus 1% sucrose) for 20 min. Finally, the excess glutaraldehyde was removed by further perfusion for 10–15 min with fixation buffer alone. All perfusion was carried out at a monitored pressure of 120 mm Hg using air pressure generated in a large vessel attached to a manometer. This is particularly critical to avoid distension in the kidney. Fetal mice were fixed using a similar regime but inserting a fine glass cannula into the left ventricle and cutting the right atrium. The tissue was excised and dehydrated for routine paraffin histology. 5-μm sections were stained according to Hsu et al. (19) with four modifications: (a) The initial blocking reagent was 1% hyperimmune rabbit anti-mouse IgG which had been absorbed with rat IgG. We found that normal rabbit serum was inadequate for preventing a nonspecific cytoplasmic staining of tubular epithelial cells. The rabbit anti-mouse IgG also blocked cross-reaction of the biotinylated rabbit anti-rat IgG second antibody reagent with endogenous mouse IgG. (b) Dulbecco's phosphate-buffered saline (PBS) was used throughout for all dilutions of reagents. (c) The peroxidase substrate, diaminobenzidine (Polysciences, U.K. Ltd.) and H_2O_2, was made up in PBS plus 10 mM imidazole, pH 7.4, as suggested by Strauss (20). The inclusion of imidazole greatly improved the intensity and localization of staining on macrophage processes. (d) All incubation times were increased as follows: first antibody, 2 h; second antibody, 1 h; and avidin-biotin-peroxidase reagent, 1 h at room temperature. The washing step after the first antibody was increased to 20 min. After staining, the sections were counterstained with Mayer's hematoxylin, dehydrated through graded ethanol baths, cleared in xylene, and mounted under coverslips using DPX. Sections were photographed using an Ilford 303 blue filter (Ilford Ltd., Ilford, UK) to increase contrast of the brown reaction product against the blue counterstain.

Results and Discussion

F4/80 specifically labels macrophages throughout the mouse kidney but the distribution is distinctly nonrandom. The highest concentration of macrophages is the medullary interstitial cell population where labeled cells with typical macrophage morphology constitute the vast majority (Fig. 1, A and B). In a longitudinal section of the medullary rays (1 B) it is clear that the interstitial cells are extensively spread and cover much of the outer surface of the collecting tubules. These cells are generally considered to be the major renal source of E-type prostaglandins as judged from studies on isolated cells and immunohistochemical localization of cyclo-oxygenase (21–25). They have not previously been identified as macrophages. Similar interstitial cells are also found throughout the cortex, though not in such numbers, in direct physical contact with the outer surfaces of the proximal and distal tubules and, in particular, the Bowman's capsule (Fig. 1, C and D). At the vascular pole of the glomerulus the epithelium of the adjacent distal tubule becomes elevated and cuboid, forming the macula densa. The macula densa interacts with specialized renin-producing cells to form the juxtaglomerular complex, which regulates glomerular capillary flow (hence ultrafiltration rate and blood pressure) in response to changes in distal tubular contents (15, 26–28).

Macrophages are always found in close proximity to the macula densa and the afferent and efferent arterioles of the glomerular capillary bed (Fig. 1, C and D).
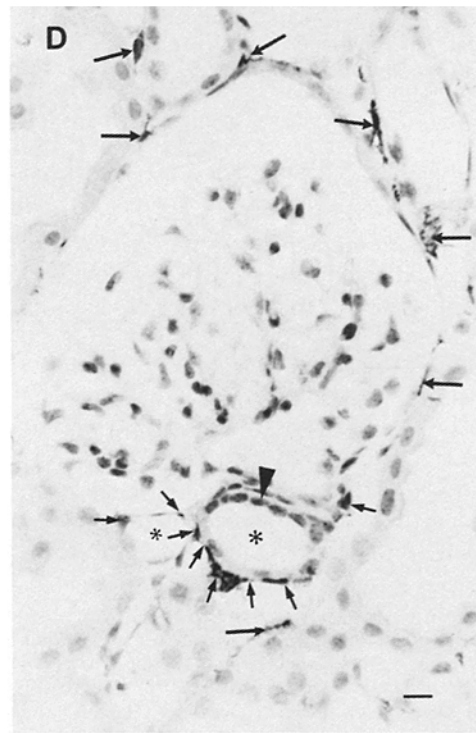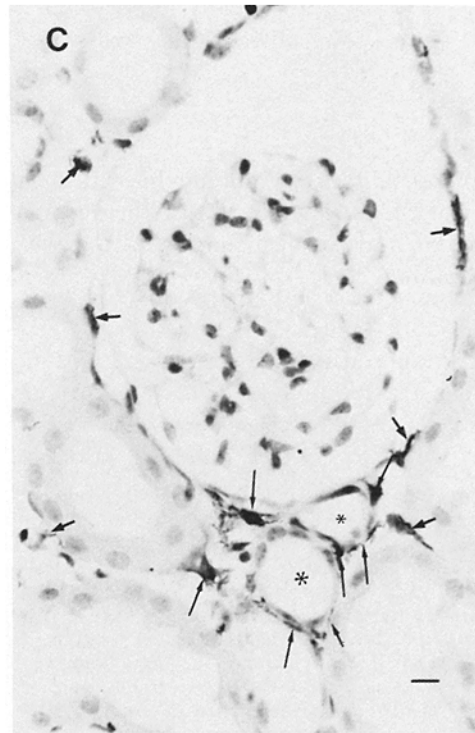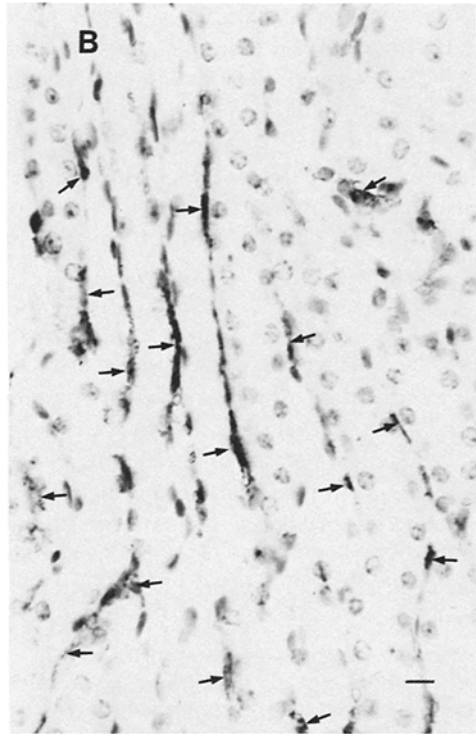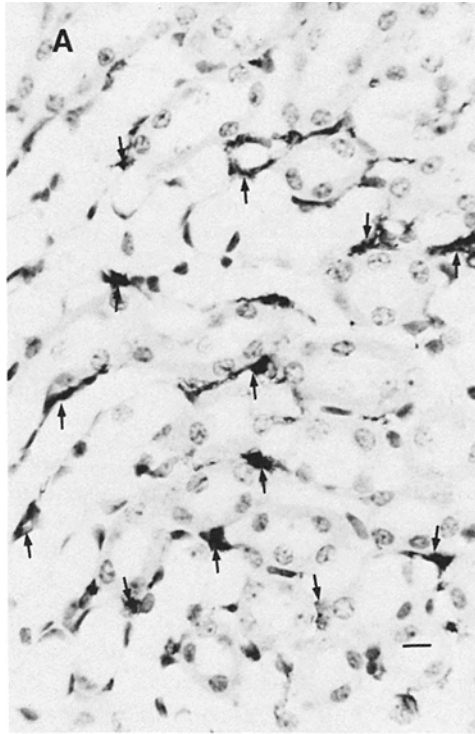
They are not equivalent to the renin-producing juxtaglomerular cells (15, 29) that are not labeled with F4/80, but must still be considered a physical part of the juxtaglomerular complex. Glomerular mesangial cells, which have at times been considered to be phagocytic (30), do not bear the antigen, which is never seen in the glomerulus itself in the healthy adult mouse (Fig. 1, C and D). In accord with the concept that mononuclear phagocytes derive ultimately from a blood precursor (monocyte) (31), F4/80 is not present in the fetal kidney before vascularization (unpublished data).

We do not know whether resident renal macrophages are influenced by their environment to express unique new functions or whether they continue to express the secretory, endocytic, and catabolic functions of macrophages in other sites (7, 10). Their anatomical location in the kidney alone is suggestive of a central function in regulation of renal physiology. Another area where macrophages may contribute is in the regulation of erythropoiesis. The recent report that some macrophage secretory functions are sensitive to hypoxia may be particularly relevant to such a function (32). The kidney is considered a major source of erythropoietin (33) and macrophages contribute to its extrarenal production (34). The possibility must now be considered that macrophages are also an important renal source of erythropoietin.

We have not yet investigated the distribution of F4/80 in various forms of nephritis. Gelfand et al. (35) have considered the binding of IgG immune complexes in renal interstitium, which probably involves Fc receptors on the macrophages identified in our studies, to be an important event in interstitial nephritis. In various models of glomerulonephritis it has been suggested that monocytes/macrophages infiltrate the glomerulus from the circulation (36–38), but the role of resident macrophages, particularly those surrounding the Bowman's capsule, must now be considered. Antigen F4/80 should provide an excellent marker for macrophage infiltration in the available mouse models of glomerulonephritis.

Summary

Macrophages have been identified in mouse kidney by immunohistochemical localization of the macrophage-specific antigen F4/80. They constitute the majority of the renal medullary interstitial cell population and are also found in contact with cortical distal and proximal tubules and Bowman's capsule. They are a physical component of the juxtaglomerular complex.

Fig. 1.   Immunohistochemical localization of antigen F4/80 in mouse kidney. No stain was observed in sections incubated with several irrelevant monoclonal antibodies in place of F4/80 in the staining procedures. Bar denotes 10 µm in all sections. (A) A cross section through medullary rays showing numerous F4/80$^+$ cells (arrows) attached to the outer surfaces of the collecting tubules and surrounding capillaries. (B) A longitudinal section through medullary rays showing the extensive spreading of F4/80$^+$ cells to cover the majority of the surface of the collecting tubules. (C) A section through a glomerulus showing the distal convoluted tubule (large asterisk) and associated arteriole (small asterisk) approaching the vascular pole. F4/80$^+$ cells (long arrows) surround most of the distal tubule and insert membrane processes between the tubule and the arteriole. Other F4/80$^+$ cells (short arrows) surround Bowman's capsule and associate with proximal tubules. (D) A section through a glomerulus shows the specialization of the epithelium of the distal tubule (large asterisk) to form the macula densa (large arrowhead). F4/80$^+$ cells in the vicinity of the vascular pole (small arrows) are closely associated with the distal tubule and the adjacent arteriole (small asterisk). Other F4/80$^+$ cells (large arrows) surround Bowman's capsule and associate with proximal tubules.

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