The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: Macrophages of endocrine organs

(pituitary/adrenal cortex/corpus luteum/testis)

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ABSTRACT Macrophages of endocrine organs have been identified by immunohistochemical localization of the macrophage-specific antigen F4/80. F4/80$^+$ cells line vascular sinuses and capillaries in anterior and posterior pituitary, adrenal cortex, corpus luteum, parathyroid, pineal gland, and islets of Langerhans. In testis approximately 20% of interstitial cells are F4/80$^+$. F4/80$^+$ cells infiltrate corpus luteum in increased numbers during luteolysis.

The mononuclear phagocyte system is a group of cells that consists of bone marrow precursors, blood monocytes, and tissue macrophages (1). F4/80, a 160-kilodalton membrane glycoprotein antigen defined by a rat monoclonal antibody, is a marker for mouse mononuclear phagocytes *in vitro* (2, 3) and *in vivo* (4–7). The present paper is concerned with immunohistochemical localization of the macrophage marker F4/80 in endocrine organs of the mouse.

METHODS

The animals used in this study were adults between 10 and 14 weeks of age. Females used in the study of the ovary were maintained in a lighting regime of 14 hr light, 10 hr dark and were chosen after displaying two consecutive 4-day estrus cycles as defined by daily vaginal smearing.

The animals were perfused through the left ventricle with heparinized saline followed by 0.5% glutaraldehyde in 1% sucrose/0.1 M sodium cacodylate as described (4). It was noted that the reliability of this method, particularly for endocrine organs, was increased if the preperfusion buffer (heparinized saline) was warmed to 37°C. Fixed tissues were excised, dehydrated through ethanol, then 100% isopropyl alcohol, cleared in ligroin (BDH), and embedded under reduced pressure in Polywax (57°C melting point, Difco). Alternatively, tissues were embedded in low-melting-point 42°C RAL paraffin wax (Stansen Scientific, Sydney, Australia), which gave slightly poorer morphology but more reliable antigen preservation. This alternative was used for testis and pancreas.

Immunohistochemical localization of F4/80 was carried out by using the method of Hsu *et al.* (8) (Vectastain kit no. PK4004, Vector Laboratories, Burlingame, CA) as modified previously (4). Staining of F4/80 was specific and was not observed when first antibody was omitted or replaced with an irrelevant rat monoclonal antibody. Photomicrographs were taken through a blue (Ilford 303) filter to increase contrast.

RESULTS

Adrenal Gland. The zona glomerulosa, the outer cortical region associated with mineralocorticoid production, con-

tains an abundant F4/80$^+$ population (Fig. 1A). The bodies of the labeled cells are wrapped around capillaries or line vascular sinuses, but membrane processes extend into the surrounding tissue so that many cells have adjacent F4/80$^+$ material. By comparison with the zona glomerulosa, the radiating sinuses of the zona fasciculata are less heavily populated with F4/80$^+$ cells. At low power they appear absent, but closer examination shows that the cells are much more extensively spread than in zona glomerulosa with decreased stain intensity (Fig. 1A). Adrenal cortical sinusoidal F4/80$^+$ cells are smaller and more flattened (in the sinus wall) than liver Kupffer cells (7). The adrenal medulla, the source of catecholamines, also contains F4/80$^+$ cells. Occasional stained cells are clearly sinusoidal, but most of the cells have no obvious vascular association (at least in the plane of section) and have long membrane processes extending between the clusters of polyhedral cells (Fig. 1B).

Pituitary Gland. The pituitary gland is composed of several functional zones, which have different F4/80$^+$ cell populations. The hypothalamus contains abundant F4/80$^+$ cells with morphology characteristic of microglia (not shown). Similar cells are present throughout the posterior lobe. The tissue is highly vascularized, and the majority of labeled cells are adjacent to capillaries and have long membrane processes (Fig. 1C). The intermediate lobe, on the other hand, is almost devoid of F4/80 antigen (Fig. 1 C and D). Finally, the anterior lobe contains an F4/80$^+$ sinusoidal/perivascular population similar in density and morphology to that of the zona glomerulosa of adrenal cortex (Fig. 1D).

Thyroid. F4/80$^+$ cells of the thyroid are of two kinds. One is a readily visible interstitial population (Fig. 1E). The second can be seen only at high power with the benefit of the color contrast between the brown stain and blue counterstain. The perifollicular F4/80$^+$ cells lie spread at the base of the follicular epithelium, but the limitations of the present technique preclude an accurate assessment of their numbers and position relative to the fine capillaries that also line the base of the follicles.

Parathyroid Gland/Islets of Langerhans/Pineal Gland. Each of these organs is highly vascularized with fine capillaries and vascular sinuses. The F4/80$^+$ cell populations present in all three are predominantly perivascular/sinusoidal (Fig. 1 F and G; pineal not shown) and are comparable in numbers to the anterior pituitary and adrenal zona glomerulosa.

Testis. F4/80 antigen is excluded from the seminiferous tubules, which are relatively poorly fixed after vascular perfusion. A population of F4/80$^+$ cells is readily visible amongst the interstitial Leydig cells (Fig. 1H). Numerically, there are approximately five larger, rounded Leydig cells for

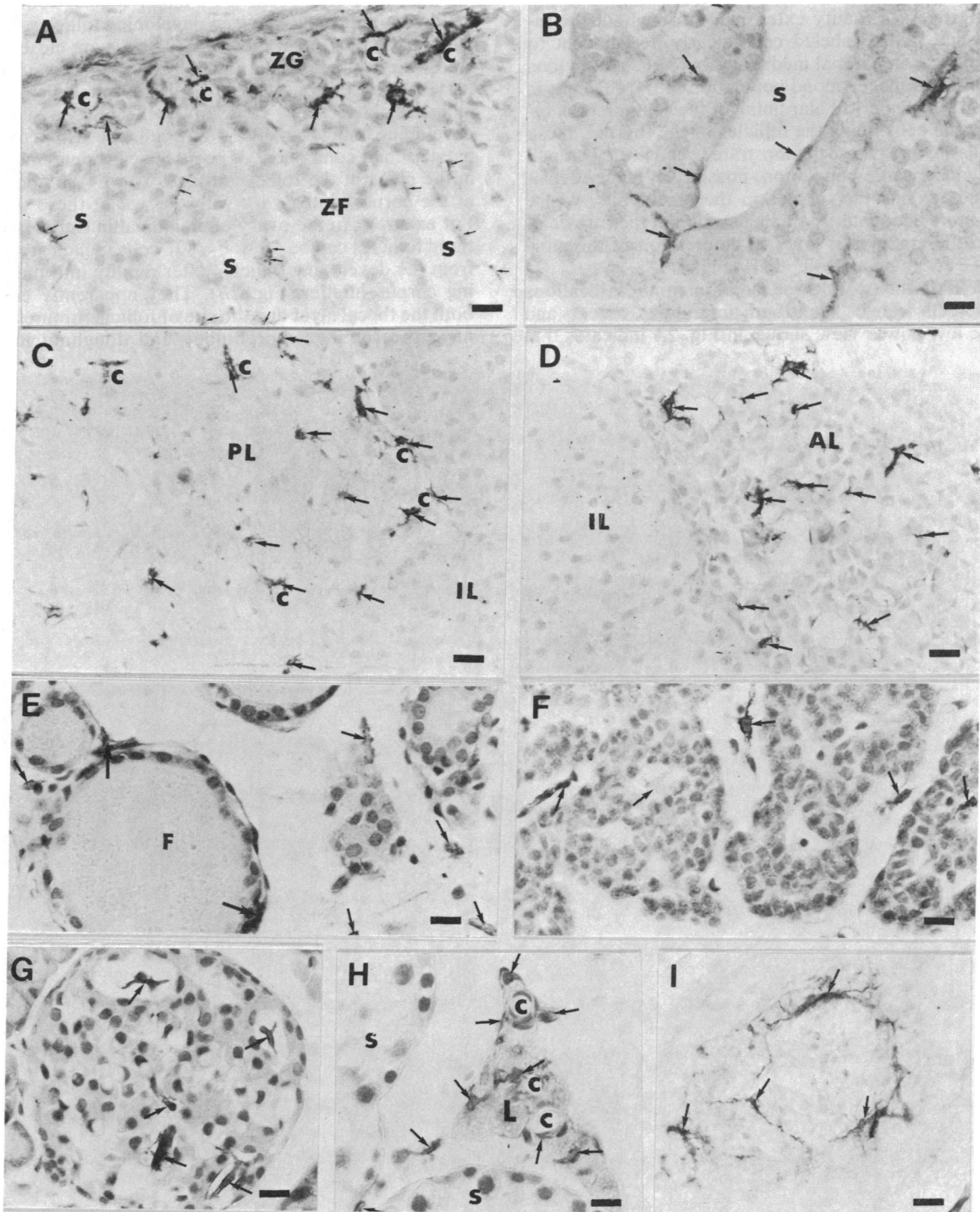‡Present address: St. Thomas's Hospital Medical School, London SE1, U.K.

FIG. 1. Immunohistochemical localization of F4/80 antigen in sections of mouse endocrine organs. (*A*) Adrenal cortex. In the outer cortex (zona glomerulosa, ZG) stellate F4/80$^+$ cells (large arrows) extend a network of processes between the steroid-secreting cells. Note the frequent association of labeled cells with capillaries (c). F4/80$^+$ cells in the inner cortex (zona fasciculata, ZF), indicated with small arrows, are less obviously stained and are spread along the walls of the radiating vascular sinuses (s). (Bar = 15 μm.) (*B*) Adrenal medulla. F4/80$^+$ cells (arrows) can be seen lining the vascular sinuses (s) and extending membrane processes throughout the tissue. (Bar = 10 μm.) (*C*) Pituitary. F4/80$^+$ cells in the posterior lobe (PL), indicated by arrows, are frequently associated with capillaries (c). The intermediate lobe (IL) is devoid of F4/80 staining. (Bar = 15 μm.) (*D*) Pituitary. F4/80$^+$ cells (arrows) are numerous in the anterior lobe (AL) but absent from the intermediate lobe (IL). (Bar = 15 μm.) (*E*) Thyroid. F4/80$^+$ cells in the thyroid are not intensely stained and contrast poorly with the blue counterstain. Two populations are indicated. Some (large arrows) are spread at the base of follicles (F); others (small arrows) are scattered in the interstitium. (Bar = 18 μm.) (*F*) Parathyroid. F4/80$^+$ cells (arrows) are predominantly perivascular or interstitial. (Bar = 18 μm.) (*G*) Islet of Langerhans. F4/80$^+$ cells (arrows) are mainly associated with vascular sinuses, which are often distended by the perfusion method of fixation. (Bar = 18 μm.) (*H*) Testis. F4/80$^+$ cells (arrows) are numerous amongst the interstitial cells of Leydig (L); many are closely associated with the numerous capillaries (c). (Bar = 18 μm.) (*I*) Epididymis. Tubules of the epididymis are better preserved than seminiferous tubules of the testis. Both possess a peritubular F4/80$^+$ population. This is a grazing section of the surface of an epididymal tubule, showing F4/80$^+$ cells (arrows) extending fine membrane processes across the surface. (Bar = 15 μm.)

each F4/80$^+$ cell in the interstitial compartment, but F4/80$^+$ membrane processes ramify extensively throughout the interstitial tissue. Some labeled cells clearly lie adjacent to capillaries but, as in adrenal medulla, most have no obvious vascular association. A second population of F4/80$^+$ cells is a component of the peritubular lining of the seminiferous tubules. Like the cells lining the follicles of the thyroid, these F4/80$^+$ cells are difficult to demonstrate because they branch extensively. The same population, possibly at higher density, continues to line the tubules in the epididymis, where fixation is more adequate. A grazing section of the surface of a tubule in the epididymis gives an impression of the morphology of peritubular F4/80$^+$ cells (Fig. 1*I*).

Ovary. Within the ovary three major anatomical locations for F4/80$^+$ cells can be identified: interstitial, thecal, and luteal. The low-power view shown in Fig. 2*A* indicates that

in the ovary F4/80$^+$ cells are a major cellular component of the interstitial tissue between developing follicles. In the interstitial compartment most F4/80$^+$ cells are closely associated with capillaries and extend processes into surrounding tissue, often in contact with cells morphologically resembling steroid-secreting cells. In the thecal layer surrounding developing follicles many F4/80$^+$ cells are also perivascular, but a second component of positive cells apparently spreads in the plane of the follicle surface. The F4/80$^+$ populations of the corpora lutea vary with the age of these structures. For example, in the ovary of an animal in estrus newly ruptured follicles can be seen. F4/80$^+$ cells, which are excluded from the developing follicle, enter rapidly into the developing corpus luteum (Fig. 2*B*). They apparently enter from both the thecal layer and the site of follicle rupture and many have the rounded morphology and doughnut nucleus of
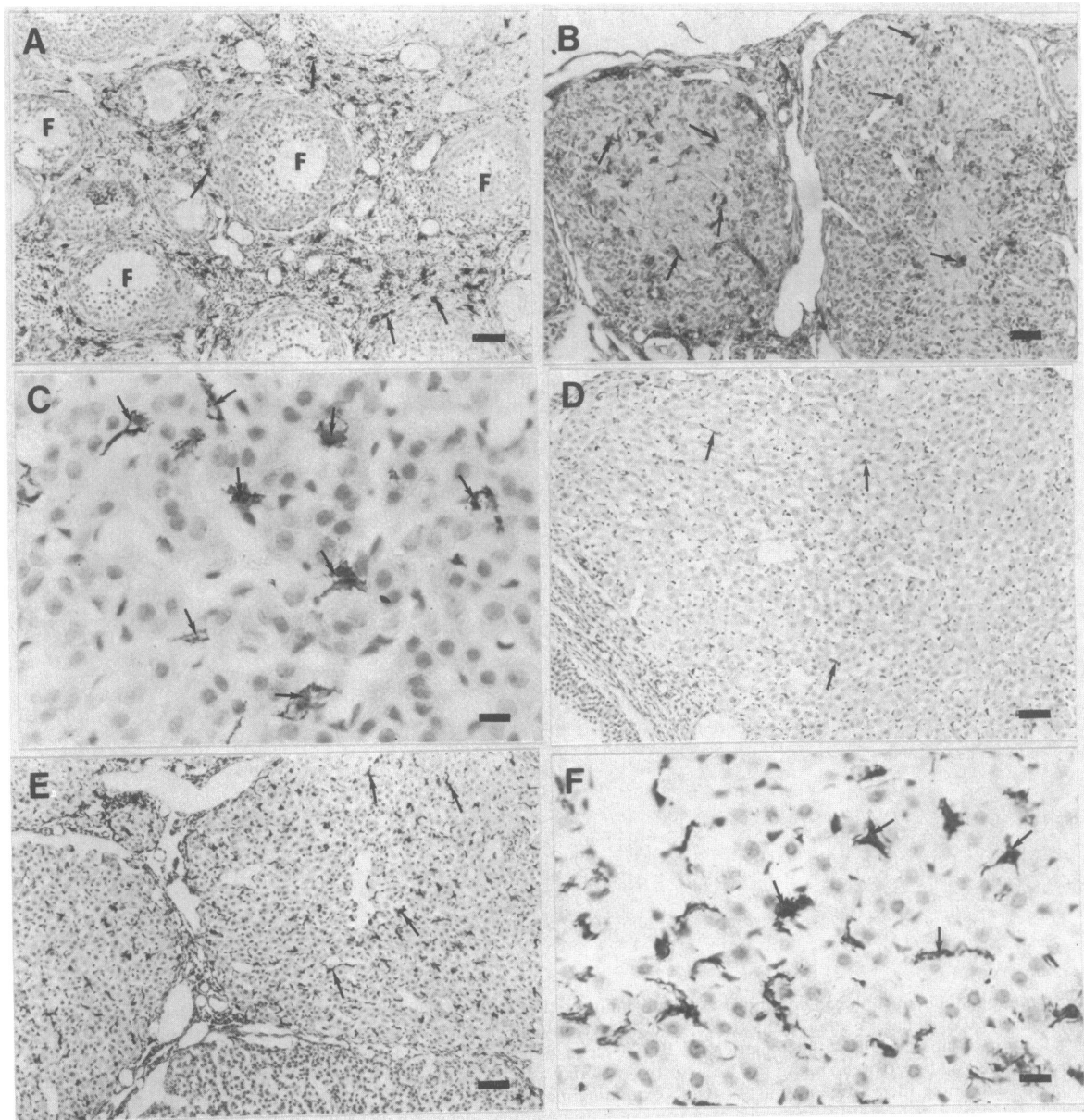

FIG. 2. Immunohistochemical localization of F4/80$^+$ antigen in sections of mouse ovary. (*A*) Low-power view of ovary in diestrus I. Note the numerous F4/80$^+$ cells (arrows indicate some examples) in the highly vascularized interstitial tissue between the developing ovarian follicles (F). (Bar = 75 μm.) (*B*) Low-power view of ruptured follicles in estrus ovary. F4/80$^+$ cells (arrows indicate examples) have already infiltrated the developing corpus luteum. (Bar = 75 μm.) (*C*) Corpus luteum, diestrus day 1. Stellate F4/80$^+$ cells (arrows) line the extensive network of vascular sinuses. (Bar = 18 μm.) (*D*) Corpus luteum, day 19 of pregnancy. F4/80$^+$ cells (arrows indicate examples) are not readily distinguished at this low power. (Bar = 75 μm.) (*E*) Corpus luteum, day 7 postpartum. In contrast to corpus luteum of pregnancy (*D*), F4/80$^+$ cells (arrows indicate examples) are a prominent cell population. (Bar = 75 μm.) (*F*) Corpus luteum, day 7 postpartum. High-power view of *E* shows numerous F4/80$^+$ cells (arrows) amongst the degenerating luteal cells. (Bar = 18 μm.)

blood monocytes. By the following day (diestrus I) the structure of the corpus luteum is well established and F4/80$^+$ cells line the vascular sinuses (Fig. 2C). This morphology is maintained through diestrus II, but by proestrus luteolysis becomes evident. This is accompanied by a further substantial influx of F4/80$^+$ cells (not shown). Older corpora lutea can be identified in subsequent estrus cycles, and by 5–6 days after estrus (diestrus I and II in next cycle) the degenerated corpus luteum contains mainly F4/80$^+$ cells.

Luteolysis is delayed when the animal becomes pregnant, and the luteal cells hypertrophy. Formation of the corpus luteum of pregnancy is not associated with further infiltration of F4/80$^+$ cells. The existing F4/80$^+$ cells are much more extensively spread in the vascular sinuses and appear less intensely stained (Fig. 2D). They resemble the cells of the zona fasciculata of adrenal cortex. After parturition the F4/80$^+$ population remains stable for 4–5 days. By day 7 postpartum, infiltration of F4/80$^+$ cells begins in association with luteolysis (Fig. 2 E and F).

There is one other circumstance in which F4/80$^+$ cells invade ovarian follicles. Throughout the estrus cycle many follicles fail to reach maturity and are lost to the process of atresia. Such atretic follicles contain numerous F4/80$^+$ cells (not shown).

DISCUSSION

F4/80 has been shown in previous studies to be a differentiation marker for macrophages in culture and *in vivo* and to be absent from any other defined cell type (4–7). In addition to expression of F4/80 antigen, F4/80$^+$ cells in endocrine organs share two features with macrophages of other organs typified by liver Kupffer cells (7). First, most have a distinctive stellate appearance with multiple extended membrane processes. Second, almost all are located in one of two categories of anatomical site, perivascular/sinusoidal and periepithelial, like the majority of macrophages in other organs (6, 7). Thus, the results presented here remain consistent with the proposal that F4/80$^+$ cells identified in tissue sections are macrophages.

Cells of the mononuclear phagocyte system display considerable functional heterogeneity (1). In addition to their phagocytic functions, macrophages possess diverse secretory products, including growth factors, neutral proteinases, and arachidonic acid metabolites (9, 10). There is a growing awareness that local hormonal interactions may be of paramount importance in the control of, for example, pancreatic islet (11), ovarian (12), and testicular (13) function. Close physical contact between F4/80$^+$ membrane processes and cells secreting steroid and peptide hormones is a common feature of the endocrine organs studied here. Although macrophages are not a numerically dominant component of the tissues (2–5% in most organs, up to 20% in testis interstitium), each cell has several membrane processes extending up to 30 μm. Thus, F4/80$^+$ macrophages form a network throughout the endocrine organs.

The corpus luteum has been the only endocrine organ studied previously with respect to macrophage function. Our results support the contention (14–17) that macrophages are involved in phagocytosis of degenerating luteal cells during normal estrus, postpartum luteolysis, and follicle atresia. Kirsch *et al.* (18) have isolated luteal macrophages and shown that they manifest most macrophage characteristics, including F$_c$ receptors, latex phagocytosis, and nonspecific esterase. These workers found that cocultivation of macrophages (peritoneal or ovarian) with luteal cells led to enhanced progesterone production, and they suggested that this trophic interaction involved direct physical contact.

Macrophages possess several biochemical activities that may be relevant to the secretion of steroid hormones. They release estradiol from its serum binding component (19), synthesize testosterone and its active 5α-reduced metabolite (20), and possess the enzyme 20α-hydroxysteroid dehydrogenase (unpublished observation).

The potential roles of macrophages in modulation of peptide hormone secretion are more speculative, but two functions are suggested from the known macrophage activities. First, macrophages may, through their arsenal of neutral proteinases and aminopeptidases (10), participate in extracellular processing of peptide hormones. For example, macrophages possess an angiotensin-converting enzyme that is induced by glucocorticoids (21). Second, macrophages are major secretors of E-type prostaglandins (9, 10), which are important regulators of peptide hormone secretion (e.g., see refs. 22 and 23). There is clearly no guarantee that macrophages from endocrine tissues identified with F4/80 possess all the biochemical activities of macrophages in other sites, such as the peritoneum. Further studies using histochemical techniques and cell isolation will be needed to assess their function.

A recent paper has described the isolation and characterization of rat testicular interstitial macrophages (24).

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