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Macrophages in Endocrine Glands, with Emphasis on Pancreatic Islets

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ABSTRACT We review here the macrophages found in endocrine tissues, placing emphasis on those residing in the islets of Langerhans of the pancreas. The islets represent the endocrine organ where macrophages have been examined in great detail and where our own studies and experience have been directed.

That macrophages normally inhabit endocrine organs became evident from the early studies in mice made in the laboratories of Siamon Gordon and David Hume (1). They used the macrophage surface marker F4/80 to examine tissue sections by immunohistochemistry and found macrophages in all endocrine organs. In most organs, the macrophages were found associated with the vessels. To note are several important examples. In the adrenal gland, the zona glomerulosa contained abundant macrophages that "wrapped around capillaries or line vascular sinuses, but membrane processes extend into the surrounding tissue." In the pituitary, they found them distributed differentially in the various areas. In the thyroid, many surrounded the follicles. In the testes, the macrophages were next to the Leydig cells and not inside the seminiferous tubules. In the ovaries, macrophages were abundant around follicles and usually surrounding the vessels.

In sum, they found three distinctive features of endocrine-resident macrophages: (i) most were associated with blood vessels; (ii) some surrounded the endocrine cells or glands, extending filopodia among them; and (iii) in some organs such as the pituitary, there were differences in the distribution depending on the anatomy.

An understanding of the important role of macrophages in the normal homeostasis of endocrine organs has come from many studies, but particularly from Jeffrey Pollard's group, many carried out in the *Csfm*$^{op/op}$ [op/op] mouse strain (2–4). ("Homeostasis" is the term used by Walter B. Cannon in discussing the *milieu interieur* of Claude Bernard: at the cellular level, we refer to those cell functions needed to maintain a tissue under steady state [5]). The op/op mouse strain has a natural null mutation in the *Csf1* gene and lacks functional colony-stimulating factor-1 (CSF-1), resulting in marked absence of macrophages in most tissues (6). CSF-1 is a protein made in several tissues that regulates the differentiation of the macrophage lineage (7). The op/op mice were first described for their osteopetrosis resulting from a lack of osteoclasts, a differentiated cell of the macrophage lineage required to remove bone matrix. The production of CSF-1 was absent from all tissues examined (7). In the initial evaluation of this strain carried out by Stanley, Pollard, and associates, a partial correction was obtained by infusing mice with CSF-1. The op/op mice also showed a number of abnormalities, particularly in endocrine glands (4). The strain is an eloquent

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example of the role that macrophages play beyond the classical scavenger and phagocytic function accentuated since their classical description by Metchnikoff that resulted in the "phagocytic theory" of immunity.

THE ISLETS OF LANGERHANS

Pancreatic islets in all species contain a resident set of macrophages. These were first considered as "passenger leukocytes," referring to blood leukocytes thought to be trapped in isolated islets. In a series of studies carried out using isolated islets for transplantation to diabetic patients, it was found that a preculture of the islets resulted in the escape of leukocytes from them; hence the conclusion that these cells derived from blood cells. These islets transplanted in certain allogeneic combinations were not rejected. The implication was that the macrophages were the target of the transplantation reaction since they expressed the proteins encoded in the major histocompatibility gene complex (MHC). These findings are extensively reviewed with appropriate references in reference 8. Studying the phagocytes in islets is important particularly because this mini-organ is the target of diabetic autoimmunity (9). Macrophages have an antigen-presenting function that is relevant in the autoimmune process.

Recent analyses have been carried out examining islets obtained from the pancreas using combinations of cell surface markers and gene expression analysis. Islets are harvested by techniques that separate them from the acinar tissue. The islets are then disrupted so individual cells can be examined. These recent studies clearly established that islets under steady-state conditions, that is, in noninflammatory situations, harbor a normal set of resident macrophages (10). These macrophages are represented by a single set identified by their display of membrane proteins typical of macrophages as well as by their expression of macrophage-specific genes.

Most of the detailed analyses of islet macrophages have been carried out in the mouse. Islets isolated from nondiabetic mice contain a small number of macrophages, usually from 5 to 10. There is an interesting correlation between the size of the islets and their number of macrophages: for example, about 5 to 10% of islets are of large size and can harbor as many as 30 macrophages. The macrophages are located next to blood vessels and in very close contact with β cells; in live image analysis of islets, the macrophages are seen extending long filopodia in between β cells, some reaching up to the edge of the islet. Moreover, filopodia also extend along the vessel wall and into the blood vessel lumen.

Of great interest are the findings showing that every macrophage takes up secretory granules from β cells (11). This uptake was substantiated by electron microscopy of islets that showed typical insulin-containing dense core granules inside the phagocytic vesicles of the macrophages. Examination of the macrophages using antibodies to insulin or insulin products confirmed the presence of insulin and insulin peptides inside the macrophage phagocytic vesicles (12). Such uptake is a normal feature of the islet macrophage found in all strains examined. The uptake requires live β cells and is unrelated to β-cell death.

Pertinent to this finding are the studies made in the NOD mouse, an inbred strain that spontaneously develops autoimmune diabetes. In most mouse colonies, the majority of NOD mice become diabetic within the 18th to 24th week of life, with islets heavily infiltrated by lymphocytes and inflammatory cells. Diabetic autoimmunity is an autoimmune disease caused by autoreactive T cells that recognize peptides derived from the intracellular processing of β-cell proteins. These peptides associate with the MHC class II (MHC-II) molecules to form the molecular complex recognized by the T cells. The MHC-II genes that confer the propensity for diabetes are very similar structurally between the NOD mouse and humans with type 1 diabetes (13, 14). In both situations, their MHC-II molecules lack a critical amino acid, an aspartic acid at the β-chain residue 57. The MHC-II peptidomes of the diabetes-propensity molecules from humans and mice are very similar. Analysis by mass spectrometry of peptides eluted from the MHC-II molecules showed them to be rich in peptides with acidic residues at their carboxy end (15). In the NOD mouse, which develops spontaneous diabetic autoimmunity, the uptake of β-cell granules has biological significance: macrophages degrade the content of the granules, incorporating some of the peptides to their MHC-II proteins to be presented to autoreactive T cells (10, 11). β Cells do not express MHC-II proteins but only MHC-I at low levels. Importantly, besides macrophages, the islets also contain dendritic cells that infiltrate at a very early stage in the process and that are essential for the development of autoimmunity, presumably in cooperativity with the resident macrophages (16). One autoantigen that is prominent both in NOD and in patients with type 1 diabetes is insulin (9, 17, 18).

The physiological role of the islet macrophages is to maintain islet homeostasis (2, 10). There is a truly symbiotic relationship between β cells and macrophages, with both influencing each other. Examination of the osteopetrotic op/op mouse is to the point: their islets are

half the size of normal and hypoinsulinemic (2, 10). Most islets lack macrophages; at the most, a few may have one or two macrophages per islet. Many op/op mice have an impaired glucose tolerance test. On the macrophage side, and in contrast to macrophages from other tissues, the islet macrophages in wild-type mice have a gene signature of activation, with expression of tumor necrosis factor and interleukin-1β and very high levels of MHC-II molecules (10). Their gene expression pattern is compatible with an M1-like activated macrophage. There is a range of genes expressed in macrophages under conditions of activation, and in broad terms they can be grouped into an M1 or M2 pattern (19). The M2 gene activation signature was defined after interleukin-4 treatment (20) and is believed to be involved in tissue repair.

The recent report from Calderon et al. made a critical analysis of pancreatic macrophages in nondiabetic mice. Islet macrophages were found during embryological development from definitive hematopoiesis (10) (Fig. 1). (For a review on recent developments in macrophage differentiation, see reference 21.) Normally in noninflammatory conditions islet macrophages show a low level of replication and are not supplied by blood monocytes. Parabiosis experiments were carried out between members of the pair having a different CD45 allele: there was no exchange between the two partners, while monocytes and lymphocytes in the spleen exchanged evenly between the two mice (10). These resident macrophages were maintained by a low level of proliferation.

Macrophages are also found in the interacinar stroma, about 10 times more than in the islets. But these stromal macrophages are very different from those in the islets (Table 1). The stromal macrophages are composed of two distinct sets based on their embryological development and by their expression of the mannose receptor, CD206, and the protein CLEC10A (CD301). One set express low levels of CD206/CD301, high levels of MHC-II molecules, and are constantly maintained from blood monocytes. This set of macrophages are dispersed throughout the stroma. A second set express high levels of CD206/CD301 and derive from yolk sac hematopoiesis. This set is enriched around the pancreatic ducts. Thus, both sets of stromal macrophages have a different anatomical localization. Both sets of stromal macrophages express an M2-like pattern of gene transcripts, in contrast to the islet macrophages, which express an M1-like pattern.

The gene expression signatures in islets and stromal macrophages are maintained with time.

An important experimental manipulation made in the report of Calderon et al. was to heavily irradiate the mice in order to deplete the islet macrophages, and to then replace them with bone marrow stem cells. After a

FIGURE 1 Pancreatic macrophages (MΦ). The pancreas contains three sets of macrophages. One set reside in the islets, labeled endocrine tissue, and have an M1-like transcriptional signature. Two sets are in the stroma in between the acinar gland, labeled as exocrine tissue. Both have an M2-like transcriptional signature. One set derives from blood monocytes; the second derives from yolk sac (YS) hematopoiesis. Reprinted from reference 10, with permission.

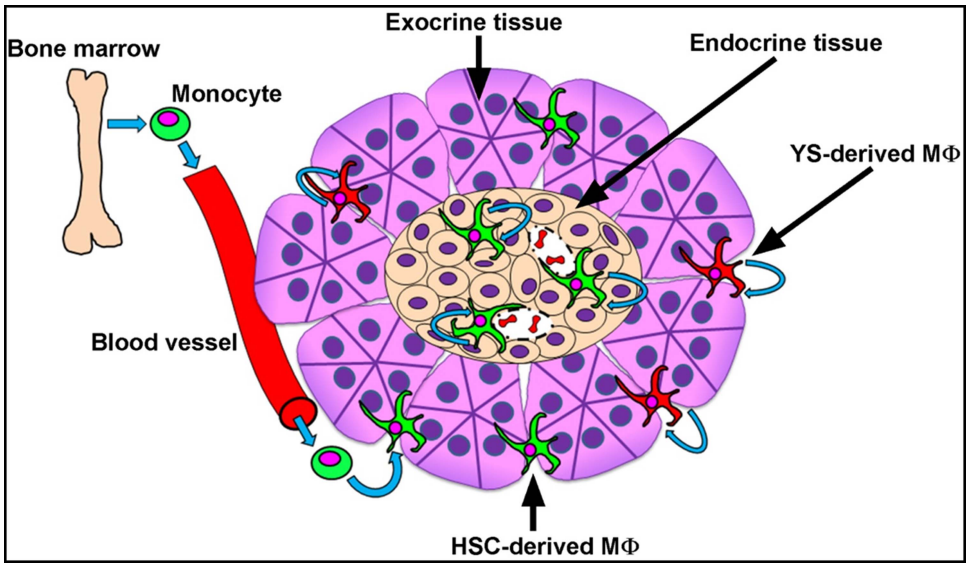

TABLE 1 Features of pancreatic macrophages

Feature	Islet	Stromal set 1	Stromal set 2
Derivation	From embryo Definite hematopoiesis	Yolk sac Hematopoiesis	Blood monocytes
Anatomy	Next to blood vessels	Enriched in peritubular areas	Dispersed
Turnover	Resident	Resident	Supplied by blood monocytes
Gene signature	M1	M2	M2
Gene signature after irradiation	M1	M2	M2
MHC-II	High	Low	High
Role	Homeostatic capture granules	Not known	Not known
Pathology	In autoimmune diabetes	Not known Role in pancreatic cancer?	Not known

period of time, the pancreas was examined. The new macrophages in islets and stroma had the same gene expression pattern as before, M1-like for those of the islets and M2-like for the stroma ones. The differences most likely are explained by the anatomy of the pancreas, its microenvironment. The Calderon et al. study concluded that the "pancreas anatomy conditions the origin and properties of the resident macrophages" (10).

REPRODUCTIVE ORGANS: TESTES AND OVARIES

Macrophages are found in the testes, where they play a major physiological role (4, 22–25). As in the pancreas, macrophages are located in two distinct anatomical sites in the mammalian testes (26). One site is in the interstitium between seminiferous tubules: the macrophages are closely associated with Leydig cells, the testosterone-producing cells. The second site is around the border of the seminiferous tubules, forming part of the peritubular capsule (26; see Comment in reference 27) (Fig. 2). At both sites, macrophages profoundly influence testicular function (4, 22–24). In the interstitium, the macrophages form tight clusters with Leydig cell (28, 29). There is a striking close contact between both cells, with cytoplasmic extensions of Leydig cells contacting the macrophages in close membrane-to-membrane contacts (29).

The macrophages are found during embryonal differentiation of the testis and have a major role in its development and early vascularization. Lineage-tracing studies indicate that the macrophages derive from early hematopoietic stem cells (HSCs) in the yolk sac (30). Such macrophages have a gene signature compatible with M2 features and express proteins typical of macrophages, like Fc receptors. When isolated, they can be shown to release cytokines (22). Studies in the op/op mouse showed markedly reduced numbers of macrophages. Accompanying this reduction was a parallel reduction in the number of Leydig cells and a low level of testosterone (4, 25). Leydig cells in the op/op mouse have a dilated endoplasmic reticulum, together with abnormal vesicles. The macrophages produce 25-hydroxycholesterol, an intermediate in the biosynthesis of testosterone that may be a substrate for its production by the Leydig cell (28).

As mentioned, macrophages are also found tightly associated with the seminiferous tubules (26). The cells are situated on the outside surface of tubules close to the stem cell niche that gives rise to spermatogonia (26). These macrophages show long filopodia extending along the surface of the tubule. They produce CSF-1, which contributes to the differentiation of the sperm cells. Macrophages from the two sites, i.e., the interstitium next to Leydig cells and on the seminiferous tubule surface, can be differentiated by their expression of the receptor for CSF-1 and expression of MHC-II molecules. Those in the interstitium have the receptor for CSF-1 but are MHC-II negative, and the reverse was shown for the macrophages of the seminiferous tubules (26). The relationship between these two sets of macrophages is not known, but the testes, like the pancreas, represent another example of the tissue conditioning its macrophage resident population while being profoundly influenced by it: a true symbiosis.

The testis has been considered an immune-privileged tissue, and the macrophages may be part of the barrier for the protection of the sperm cells (reviewed in references 31 and 32). Whether the macrophages have an antigen-presenting function in autoimmune orchitis or autoimmune aspermatogenesis has not been established. In experimental autoimmune orchitis, in which the animal is immunized with testes antigen, an initial reaction is at the surface of the seminiferous tubules (33). The thinking is that the macrophages at that site contain antigens derived from spermatogonia and may have a presenting function for autoreactive T cells.

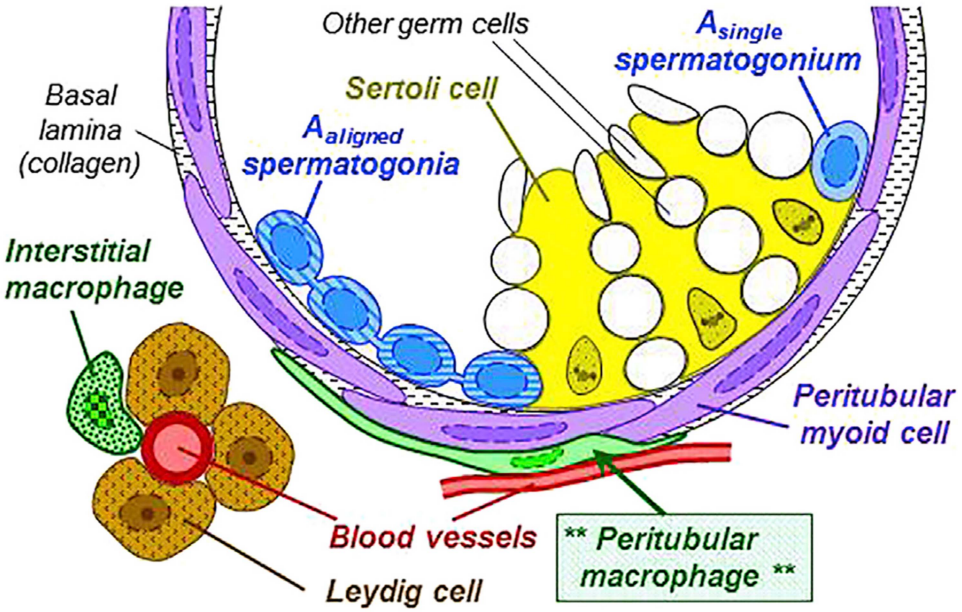

FIGURE 2 Testicular macrophages. The macrophages in the testes are found in two locations. One set are in the interstitium, forming a cluster with Leydig cells. The second set surround the seminiferous tubules—the peritubular macrophages. Reproduced from reference 27, with permission.

Macrophages are also found in the ovaries (4, 34). They are situated at different sites within the interstitial tissue that surrounds the ovarian follicles but never inside them. CSF-1 is made by cells in the granulosa layer of the follicle and has been thought to have a role in maintaining the macrophage population (4). During ovulation, the number of macrophages increases in the theca layer outside of the follicle. After the ovum is released, macrophages are prominent in the corpus luteum (35–37). The op/op mouse has defective ovarian function, ascribed in part to the profound reduction in macrophage numbers (4, 38). Estrous cycles are much longer and the degree of ovulation is reduced. In brief, as in the other endocrine organs, the macrophage modulates the function of the organ (3, 4, 39).

OTHER ENDOCRINE ORGANS

As reported by Gordon and Hume and collaborators (1), macrophages also are found in adrenal glands, the thyroid, and the pituitary. Analyses of these tissue-resident macrophages in terms of their role and embryonic derivation have yet to be done. In the adrenal, the macrophages are found in both cortex and medulla, although the number in each layer varies. Macrophages are reported to extend long projections between the endocrine cells and localizing close to blood vessels (1). Addison's disease is a rare autoimmune disease that results in the destruction of the adrenal cortex. Most patients have autoantibodies to steroid 21-hydroxylase, an intracellular endoplasmic reticulum protein enzyme involved in steroid biosynthesis (40). CD4 and CD8 T cells directed to peptides of the enzyme have been reported (41). Although not examined, it is likely that the macrophages are responsible for the uptake of the intracellular material and participate in the initiation of the autoimmune process.

Concerning the thyroid, few studies have examined macrophages under steady-state conditions. Macrophages have been detected in the pituitary gland. Their distribution varies, with the greatest number in the anterior and posterior lobes, and they are practically absent in the intermediate lobe (1, 34). The hypothalamus-pituitary-gonadal axis is markedly defective in the op/op mouse (4), but the nature of the macrophage involvement has not been examined.

FINAL COMMENT

Of the various endocrine organs, the pancreatic islets and the testes are the two in which macrophages have been examined most extensively. Three important results have come out of their analysis. The first concerns the different gene signatures among the macrophages.

The studies in the pancreas, where those in the interacinar stroma differ from those in the islets, are particularly informative and point to the microenvironment controlling the biological response of the macrophage. A second important conclusion is that the endocrine cells require the trophic function of the macrophage. This requirement was shown best through the examination of the op/op mouse. These mice show defective islets and testes function. It is also apparent that macrophages also influence ovarian function and the pituitary-gonadal axis. However, no very detailed examinations of the macrophages in the latter two sites have been carried out. The conclusion is that there is a striking symbiosis between the endocrine cells and the macrophages, each deeply influencing each other. Finally, macrophages take up products of the surrounding endocrine cells. It is likely that these are the molecules that influence the behavior of the macrophage. As we discussed for pancreatic islets, the macrophages take up whole dense core granules that contain not only insulin, about 3×10^5 molecules per granule, but other bioactive molecules such as ATP and granins.

Finally, it is important to note that endocrine organs are among the most frequent targets for autoimmunity, and this needs to be explained. Having macrophages expressing high levels of MHC-II plus containing the molecules responsible for the autoimmune reaction places them as a central player.

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