MINIREVIEW

Physiologic Interactions Between Macrophages and Leydig Cells

JAMES C. HUTSON[1]

Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas 79430

The purpose of this minireview is to present information concerning the morphologic and functional relationship between testicular macrophages and Leydig cells. Although data concerning the negative influence of macrophage-derived products on testicular Leydig cells exist, this review is focused on the stimulatory influences thought to be involved in the physiologic interactions between these two diverse cell types. Exp Biol Med 231:1–7, 2006

Key words: testicular macrophage; 25-hydroxycholesterol; 25-hydroxylase; Leydig cells

Introduction

Macrophages primarily reside in the connective tissue of most organs, where they phagocytose bacteria and signal lymphocytes of impending infections. They also recognize malignant or virally infected cells and then interact with lymphocytes in the killing of these cells. Phagocytic cells appear early in the phylogenetic scale, which emphasizes the importance of these functions. Given this long-standing existence and wide anatomic distribution, it is not surprising that they have acquired the ability to interact with cells outside the immune system (1). The purpose of this minireview is to present a brief overview of one of the paracrine interactions that exists between macrophages of the testes and neighboring Leydig cells.

[1] To whom correspondence should be addressed at Department of Cell Biology and Biochemistry, 3601 4th Street, Texas Tech University Health Sciences Center, Lubbock, TX 79430. E-mail: jim.hutson@ttuhsc.edu

1535-3702/06/2311-0001$15.00

Life Cycle and Population Dynamics

Cells possessing markers for monocyte/macrophages appear in the testicular interstitium near the time of birth in rats and, subsequently, grow in size and number as they differentiate and populate the testis (2, 3). When newborn rats are given human chorionic gonadotropin for 8 to 10 days to precociously stimulate neighboring Leydig cells, the macrophage population doubles while those of the liver remain unchanged (4). This effect is not due to the release of testosterone by Leydig cells in response to human chorionic gonadotropin (hCG) because Casodex, an antiandrogen, did not block this action, nor did testosterone injections mimic the effects of hCG (5). Although it seems likely that the effects of hCG are indirectly mediated by Leydig cells, it has been shown that placental macrophages express a variant type of luteinizing hormone (LH)/hCG receptor that is missing exon 9 (6). It is thought that this may facilitate the local metabolism of hCG by macrophages in the placenta villi. Animals treated with ethylene dimethane sulfonate (EDS) to kill Leydig cells and supplemented with testosterone had significantly fewer testicular macrophages than control animals (7), which further demonstrates the importance of Leydig cells (but not testosterone) in maintaining the normal number of macrophages in the testis. It has also been shown that the effects of Leydig cells on the number of macrophages are not indirectly mediated by way of the seminiferous epithelium (8). Proliferation of macrophages, as observed in all these experimental studies, is most likely a physiologic phenomenon because proliferation of macrophages also occurs during postnatal maturation, at a time when gonadotropin levels are rising (5, 9).

Lipopolysaccharide, a component of the cell wall of gram-negative bacteria, stimulates monocytes to migrate into the testis (10) rather than stimulating proliferation of existing macrophages. This suggests that different mecha-

nisms may be involved in expanding the macrophage population in the testis following an infection and/or during immune activation than during the normal postnatal developmental processes. It is interesting that the number of both testicular macrophages and Leydig cells increases from birth to adulthood at a relatively constant ratio (11, 12), which supports the concept that these two cell types are functionally coupled. Using several macrophage-specific markers, the adult population has been shown to be heterogeneous in rats (8, 10, 13) and mice (14). The functional significance of these subpopulations is yet to be understood. In the adult rat testis, 81% of the OX1-positive leukocytes are macrophages (express ED1 and/or ED2; Ref. 7). F4/80 is a marker commonly used to identity mouse testicular macrophages and has been used to identify those of the testis (15). Vital dyes such as trypan blue, carmine red, and a dipeptide have also been used to label macrophages *in vivo* (16). In this regard, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) should be used rather than trypan blue for assessing viability *in vitro* because macrophages readily phagocytose trypan blue with prolonged exposure (17).

Structure

Testicular macrophages of all mammalian species studied thus far exist only in the interstitial compartment under normal conditions (1, 18). In birds, during testicular regression, and in mammals, under pathologic conditions where germ cells have died, macrophages can enter the seminiferous tubules to phagocytose nonviable cells. When stained with hematoxylin-eosin, it is difficult to distinguish macrophages from Leydig cells in paraffin sections viewed with the light microscope. However, when plastic sections are stained with toluidine blue, the macrophages are much less densely stained than Leydig cells and are, thereby, very easily identified (11). The cytoplasm of macrophages is also much paler in well-preserved samples for electron microscopy (19–21). Unfortunately, in poorly preserved samples, a subpopulation of Leydig cells also stains lightly, resembling macrophages (19). Typical of macrophages in most organs, testicular macrophages have an indented nucleus, numerous lysosomes, and residual bodies that are polymorphic. Microvilli and cytoplasmic vesicles are also commonly observed. In rats testicular macrophages are round, but they are more ovoid in dogs and guinea pigs (22) and are heterogeneous in shape in the mouse (23). The most intriguing ultrastructural features of testicular macrophage are the digitations. These structures exist at sites where macrophages and Leydig cell are in direct contact and are comprised of long microvillus-like Leydig-cell processes that are inserted within coated pits of the macrophage (19, 20). There are numerous coated vesicles near these structures within the macrophage cytoplasm. Digitations form between postnatal Days 20 and 30 in rats, a time just before the surge in testosterone that occurs at puberty (21). Macrophages are not known to form these specialized areas of contact with any other cell type (21).

Effects of Macrophages on Leydig Cells

Because macrophages are closely associated with Leydig cells and are known to secrete a wide variety of substances that signal other cell types at multiple anatomic locations, the author initially proposed the hypothesis that they may secrete a factor that also influences testosterone secretion by Leydig cells. The first approach was to develop a culture system for rat testicular macrophages (24) with recent modifications (25) to test this hypothesis *in vitro*. Similar to Leydig-cell isolation procedures, this method employs collagenase dispersion of the testes, which results in a fraction that quickly sediments at unit gravity containing primarily seminiferous tubules and a supernatant containing Leydig cells, macrophages, endothelial cells, fibroblasts, peritubular cells, and other cells of loose connective tissue. When the cells of the collagenase supernatant are collected by centrifugation and resuspended in culture medium, the macrophages within this heterogeneous cell mixture will attach to culture vessels within 5 to 10 mins, allowing other cell types to be rinsed away by swirling and then removing the medium three to four times (most other cell types require 15–30 mins to firmly attach). It is important to coat the dishes with bovine serum albumin before plating to reduce adherence of contaminating cells. It is also important that the culture medium used to attach and wash the cells be preacclimated in the CO_2 incubator before use. This procedure yields a population of macrophages that are more than 95% pure (1). Cultured testicular macrophages expressed Fc receptors, phagocytosed inert particles, and pathologic bacteria; expressed macrophage-specific markers; and released superoxide anion, indicating that they are authentic macrophages (1, 26, 27). However, the protein profile, as visualized using two-dimensional gel electrophoresis, was dramatically different than the profile obtained from macrophages of the peritoneal cavity, which indicates that they have acquired a tissue-specific phenotype (28).

Using the differential attachment culture model, rat Leydig cells grown in testicular macrophage-conditioned medium were found to secrete more testosterone than control Leydig cells in a dose- and time-dependent manner (29). The factor responsible for this effect was found to be ether extractable, resistant to chymotrypsin, heat stable, and bound to dextran-coated charcoal, which indicates that it was a small lipophilic compound (25). The approach for identifying this lipophilic factor, originally called macrophage-derived factor, took advantage of the finding that it was produced by peritoneal macrophages as well as testicular macrophages. This allowed many more cells to be isolated, thereby yielding large volumes of conditioned medium (30). Twenty liters of conditioned medium were extracted in two batches from rat peritoneal macrophages

with ether, and the residue was sequentially chromatographed from the organic phase through three HPLC columns: C_{18}, silica, and finally a cyano column. A single peak of bioactivity was obtained with each column. This final bioactive peak from the cyano column was subjected to gas chromatography on a DB-5 column and, again, a single peak was obtained using total ion mass spectrometry (MS) for detection. When this peak was analyzed by MS, it exhibited a molecular weight of 402 with a fragmentation pattern characteristic of oxysterols. When a series of oxysterols was subjected to the same three HPLC columns and then gas chromatography/mass spectrometry, only 25-hydroxycholesterol had the same elution time by all three HPLC columns and the same fragmentation pattern by MS. When this preparation was further analyzed by nuclear magnetic resonance spectroscopy, the assigned resonance signals indicated that the compound was 25-hydroxycholesterol. The secretion of 25-hydroxycholesterol was not restricted to cultured macrophages as it was also found in washes of normal rat testis (30). We also found that human macrophages produce 25-hydroxycholesterol, which indicates that this phenomenon is not specific to rodents and has potential clinical relevance (31).

The pathway for conversion of free cholesterol to testosterone in Leydig cells is well established. Cholesterol is delivered to the cholesterol side-chain cleavage system located on the inner mitochondrial membrane for the initial enzymatic steps of steroidogenesis. Because cholesterol is very nonpolar, it is assisted to the side-chain cleavage enzymes by the steroidogenic acute regulatory (StAR) protein (32). Cholesterol is then hydroxylated at the 20 and 22 positions, yielding 20α,22(R)-hydroxycholesterol (33). The side chain is then cleaved between these hydroxylated sites yielding pregnenolone, which is further metabolized to testosterone through higher-capacity enzyme systems in the cytosol. The present findings indicate that macrophages have the potential to provide an alternate pathway for steroidogenesis, which bypasses the StAR-dependent delivery of cholesterol to the side-chain cleavage system by offering 25-hydroxycholesterol as a direct substrate for side-chain cleavage. The rate-limiting step for this paracrine pathway would, therefore, be the regulation of biosynthesis of 25-hydroxycholesterol in the macrophage. A 25-hydroxylase has been described (34) and has presented in both testicular and peritoneal macrophages (35).

Although the most likely fate of testicular macrophage–derived 25-hydroxycholesterol is conversion to testosterone as previously described, this oxysterol has also been shown to act as a signaling molecule in a wide variety of cell types. For example, it stimulates sphingomyelin synthesis in Chinese hamster ovary cells (36), inhibits macrophage and lymphocyte functions (37), stimulates the accumulation of intracellular calcium in smooth muscle cells (37), inhibits growth of tumor cells (38), induces apoptosis (39) and eicosanoid production (40) in endothelial cells, and regulates cholesterol metabolism (41–43). Along with other sterols, 25-hydroxycholesterol has been shown to increase StAR expression in MA-10 and Y1 cells (44, 45), but elicits no similar effect in primary cultures of rat Leydig cells (46). The cytotoxic effects of 25-hydroxycholesterol are far less pronounced in Leydig cells than in other cell types (35). Clearly, more work is needed to determine if nonsubstrate effects of 25-hydroxycholesterol play a significant physiologic role in Leydig cell development and/or function.

Several lines of evidence suggest that the paracrine interactions between macrophages and Leydig cells is physiologically relevant. First, the amount of 25-hydroxycholesterol produced by cultured testicular macrophages (10.29 fg 25-hydroxycholesterol/cell/hr) is sufficient to provide a significant amount of substrate for Leydig cells (30). A single rat testis has approximately 15 million macrophages (11). At 10.29 fg 25-hydroxycholesterol/cell, these macrophages would produce 154 ng 25-hydroxycholesterol in 1 hr. Even if only a fraction of this 25-hydroxycholesterol becomes testosterone, it would represent a physiologically relevant portion of the substrate used for testosterone produced by Leydig cells. It should be noted that the amount of 25-hydroxycholesterol produced *in vitro* may be different to that produced *in vivo*, and Leydig-cell responsiveness may also be quite different in these two environments. In addition, the volume into which macrophages secrete 25-hydroxycholesterol is obviously different *in vivo* compared with *in vitro*. However, because macrophages are in direct contact with the Leydig cells, it is likely that Leydig cells are exposed to much of the 25-hydroxycholesterol produced by these cells. The rate of turnover of 25-hydroxycholesterol, as well as the degree of conversion of 25-hydroxycholesterol to testosterone compared to 20,22-hydroxycholesterol under normal physiologic conditions are yet to be determined. Additional evidence that the stimulatory influence of macrophages on Leydig cells is physiologically relevant is that testosterone levels have been shown to be less, and animals become infertile, when macrophages are removed from the testis by experimental (47, 48) or genetic (49) approaches. In the experimental models, liposome-encapsulated toxins have been injected into the testis that kill macrophages by apoptosis (50, 51). Testosterone levels in the depleted testis are far lower than in the untreated control testis of the same animal. It has been shown that Leydig cells are not directly affected by these liposomes or toxins (52). In the genetic model, macrophages are absent because the animals produce a mutant form of colony stimulating factor-1, a growth factor responsible for the production of monocytes/macrophages (53). Although the effect of macrophage depletion in this model appears to be primarily mediated by decreased circulating levels of LH, the data also support a local macrophage-derived effect (49, 54, 55). As previously mentioned, the presence of 25-hydroxycholesterol in the testis under normal physiologic conditions makes it possible for it to be involved in the physiologic regulation of Leydig cells (30). It has also been shown that testosterone inhibits

the production of 25-hydroxycholesterol at the transcriptional level, which suggests that a negative feedback loop exists for this paracrine interaction (as is present for most endocrine systems; Ref. 56). Final evidence for a physiologic role for this oxysterol is that maturing Leydig cells treated with 25-hydroxycholesterol *in vitro* express much more 3β-hydroxysteroid dehydrogenase than untreated cells (57). Because oxysterols have been shown to function as signaling molecules with important roles in lipid metabolism, (41–43) it seems possible that they also act on Leydig cells through similar nonsubstrate mechanisms to regulate their postnatal maturation. Because macrophages are not found in the testis during prenatal development (58), it seems unlikely that they play a direct role in embryonic or fetal development of Leydig cells.

Controversy arose following our early studies that demonstrated a stimulatory effect on Leydig cells in that some laboratories found that Leydig cells produced less testosterone when grown in a macrophage-conditioned medium (1). These inhibitory effects were primarily due to the production of nitric oxide (NO), TNFα, and/or interleukin-1 by testicular macrophages, all of which have potent negative effects on steroidogenesis (59). It is likely that variations in the methods used to prepare macrophages may explain these discrepancies because the presence of endotoxin (LPS) and collagenase are known to make major differences in their secretory pattern (60–62). This is an important consideration because more recent procedures using elutriation, density-gradient sedimentation, and/or binding to opsonized particles to isolate testicular macrophages have also been described (63–65). The effects of these various procedures on the secretory profile of testicular macrophages is unknown. It is likely that the cytokines produced by testicular macrophages play important roles during pathologic conditions involving immune activation (66). Although it is possible that they also play roles in the physiologic processes of the testis, it seems unlikely because fertility persists following the deletion of many of these cytokines and/or their receptors (67–70), and most of the cytokines are either not expressed or are expressed at very low levels by testicular macrophages under physiologic conditions (66, 71). Excellent reviews concerning these inhibitory factors are available (72–75).

Cooperation between two different cell types for metabolism of steroids to their final active form is an important and conserved theme in reproductive biology. For example, theca internal cells produce androgens that are subsequently aromatized by granulosa cells to estradiol (76, 77). Similarly, androgens from Leydig cells are aromatized by Sertoli cells to estradiol (77), and androgens can be 5α-reduced by target cells yielding a more active compound (78). Thus, passage of 25-hydroxycholesterol from macrophages to neighboring Leydig cells for conversion to testosterone may be an additional example of how evolution has reproduced this most interesting theme.

Unanswered Questions

What Portion of the Total Testosterone Made by Leydig Cells Comes from Macrophage-Derived 25-Hydroxycholesterol? Depending on the species, Leydig cells acquire cholesterol from high density lipoproteins (HDL), low density lipoproteins (LDL), and/or *de novo* biosynthesis (79). As previously discussed, cholesterol is converted to 20,22-hydroxycholesterol and then pregnenolone by the cholesterol side-chain cleavage system. The delivery of cholesterol to the mitochondria where this enzyme system resides is facilitated by StAR, a protein under the regulation of LH (32). 25-hydroxycholesterol bypasses mechanisms requiring StAR and gains unassisted access to the side-chain cleavage system where it is also converted to pregnenolone and, ultimately, testosterone by the previously described mechanisms for 20, 22-hydroxycholesterol (46). The portion of testosterone produced from 25-hydroxycholesterol compared to 20, 22-hydroxycholesterol is yet to be determined. When the 20,22-hydroxycholesterol pathway is blocked, as in StAR knockout animals, there is still sufficient testosterone to maintain spermatogenesis, which suggests that alternate pathways are important. The author hypothesizes that the testosterone produced under these conditions is derived in part from 25-hydroxycholesterol. This is supported by the observation that Leydig cell lines, such as the MA-10 cell, produce very little steroid under basal conditions. This hypothesis is also supported by the finding that removal of macrophages from one testis causes it to secrete less testosterone than the opposite testis, even though the Leydig cells without macrophages are still under the regulation of LH and StAR-mediated sources of oxysterol for conversion.

What Regulates the Production of 25-Hydroxycholesterol? As previously mentioned, testosterone decreases the number of 25-hydroxylase transcripts and production of 25-hydroxycholesterol by cultured testicular macrophages. The author hypothesizes that this represents a negative feedback loop. There have been no reports of signals that increased the production of 25-hydroxycholesterol by macrophages. Additional studies are clearly needed to determine if production of this oxysterol is positively regulated.

What Is the Role of Macrophages and/or 25-Hydroxycholesterol in Leydig Cell Development? Treatment of immature Leydig cells *in vitro* with 25-hydroxycholesterol dramatically increased the histochemical reaction for 3β-hydroxysteroid dehydrogenase. It is interesting to note that the number of transcripts for 25-hydroxylase are highest in macrophages taken from animals at 10 days of age and that testicular macrophages from immature animals produced 25-hydroxycholesterol (57). Both findings indicate that this oxysterol has the potential to be a major stimulator of Leydig cell development and

maturation. Effects on parameters other than 3β-hydroxysteroid dehydrogenase have yet to be investigated.

What Is the Role of Leydig Cells in Regulating Macrophage Numbers, Differentiation, and Function? Gonadotropin administration to newborn rats results in an increased number of macrophages in the testis. Although the number of macrophages in the liver did not increase, it is not known if macrophage populations in other parts of the animal were influenced. The mechanisms mediating this phenomenon are completely unknown. It is interesting that functional hCG receptors have been found in the female reproductive tract (6, 80). The author speculates that the purpose of this is to facilitate the local metabolism of hCG by macrophages in the villi. It seems possible that macrophages of the testis could also express hCG/LH receptors and serve a similar function of scavenging LH released from Leydig-cell receptors. It would be fascinating if this were part of the role of the digitations that exists between these two cell types. It is also possible that these effects are indirectly mediated by stimulating Leydig cells to release pertinent factors. Although macrophage-inhibitory factor-1 appears not to be involved in this process, monocyte chemoattractant protein-1 may be involved (81).

Are Testicular Macrophages Truly Bone Marrow Derived and What Is the Significance of the Subpopulations? To date, all macrophages that appear in the connective tissue either migrated in from the blood as monocytes or are a result of the proliferation of these initially migrating cells. Although testicular macrophages are known to divide during postnatal maturation of the testis, it is not known if the original cells were derived from the bone marrow. Regardless of their origin, it will be interesting to determine the factors in the local testicular environment that induce this unique tissue-specific phenotype.

What Is the Function of the Digitations? Because these structures do not form between macrophages and any cell type other than Leydig cells, it seems that they must be specifically involved in functions specific to the testicular phenotype. It seems possible that these structures function as either anchors and/or sites of molecular exchange. 25-hydroxycholesterol has been observed in the membrane of macrophages (82). The author proposes that macrophage induce apoptosis in adjacent lymphocytes through such mechanisms. No similar studies have been conducted in the testis to determine the localization of 25-hydroxycholesterol in testicular macrophages and/or to determine if this oxysterol plays a similar cytotoxic role in the elimination of interstitial cells during gonadal maturation. Although little has been done to understand the role of the digitations, it is speculated that they lie at the heart of this most interesting encounter between a very versatile cell of the immune system and the most important endocrine cell of the testis.

Summary

Macrophages may be the most versatile of all cell types, with immune and nonimmune functions proposed across many organ systems. Within the testis, they play important roles during immune activation (when cytokines are released and steroidogenesis is inhibited by traditional cytokines) and normal physiologic interactions with Leydig cells (when development is stimulated and the production of testosterone is promoted by the secretion of 25-hydroxycholesterol). Additional studies of this paracrine interaction between macrophages and Leydig cells may provide valuable insight for the treatment of infertility and/or the development of more effective male contraceptive measures.

1. Hutson JC. Testicular macrophages. Int Rev Cytol 149:99–143, 1994.
2. Hutson JC. Changes in the concentration and size of testicular macrophages during development. Biol Reprod 43:885–890, 1990.
3. Raburn DJ, Coquelin A, Reinhart AJ, Hutson JC. Regulation of the macrophage population in postnatal rat testis. J Reprod Immunol 24: 139–151, 1993.
4. Raburn DJ, Coquelin A, Hutson JC. Human chorionic gonadotropin increases the concentration of macrophages in neonatal rat testis. Biol Reprod 45:172–177, 1991.
5. Raburn DJ, Coquelin A, Reinhart AJ, Hutson JC. Regulation of the macrophage population in postnatal rat testis. J Reprod Immunol 24: 139–151, 1993.
6. Sonoda N, Katabuchi H, Tashiro H, Ohba T, Nishimura R, Minegishi T, Okamura H. Expression of variant luteinizing hormone/chorionic gonadotropin receptors and degradation of chorionic gonadotropin in human chorionic villous macrophages. Placenta 26:298–307, 2005.
7. Wang J, Wreford NG, Lan HY, Atkins R, Hedger MP. Leukocyte populations of the adult rat testis following removal of the Leydig cells by treatment with ethane dimethane sulfonate and subcutaneous testosterone implants. Biol Reprod 51:551–561, 1994.
8. Meinhardt A, Bacher M, Metz C, Bucala R, Wreford N, Lan H, Atkins R, Hedger M. Local regulation of macrophage subsets in the adult rat testis: examination of the roles of the seminiferous tubules, testosterone, and macrophage-migration inhibitory factor. Biol Reprod 59:371–378, 1998.
9. Schlatt S, de Kretser DM, Hedger MP. Mitosis of resident macrophages in the adult rat testis. J Reprod Fertil 116:223–228, 1999.
10. Gerdprasert O, O'Bryan MK, Muir JA, Caldwell AM, Schlatt S, de Kretser DM, Hedger MP. The response of testicular leukocytes to lipopolysaccharide-induced inflammation: further evidence for heterogeneity of the testicular macrophage population. Cell Tissue Res 308: 277–285, 2002.
11. Hardy MP, Zirkin BR, Ewing LL. Kinetic studies on the development of the adult population of Leydig cells in testes of the pubertal rat. Endocrinology 124:762–770, 1989.
12. Ariyaratne HB, Chamindrani Mendis-Handagama S. Changes in the testis interstitium of Sprague Dawley rats from birth to sexual maturity. Biol Reprod 62:680–690, 2000.
13. Hutson JC. Changes in the concentration and size of testicular macrophages during development. Biol Reprod 43:885–890, 1990.
14. Itoh M, De Rooij DG, Jansen A, Drexhage HA. Phenotypical heterogeneity of testicular macrophages/dendritic cells in normal adult mice: an immunohistochemical study. J Reprod Immunol 28:217–232, 1995.
15. Hume DA, Halpin D, Charlton H, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical

localization of antigen F4/80: macrophages of endocrine organs. Proc Natl Acad Sci U S A 81:4174–4177, 1984.
16. Otto C, Bauer K. Dipeptide uptake: a novel marker for testicular and ovarian macrophages. Anat Rec 245:662–667, 1996.
17. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47:936–942, 1987.
18. Frungieri MB, Calandra RS, Lustig L, Meineke V, Kohn FM, Vogt HJ, Mayerhofer A. Number, distribution pattern, and identification of macrophages in the testes of infertile men. Fertil Steril 78:298–306, 2002.
19. Christensen AK, Gillim SW. The correlation of fine structure and function in steroid-secreting cells, with emphasis on those of the gonads. In: McKerns KW, Ed. The Gonads. New York: Appleton-Century-Crofts, pp415–488, 1969.
20. Miller SC, Bowman BM, Rowland HG. Structure, cytochemistry, endocytic activity, and immunoglobulin (Fc) receptors of rat testicular interstitial-tissue macrophages. Am J Anat 168:1–13, 1983.
21. Hutson JC. Development of cytoplasmic digitations between Leydig cells and testicular macrophages of the rat. Cell Tissue Res 267:385–389, 1992.
22. Miller SC. Localization of plutonium-241 in the testis: an interspecies comparison using light and electron microscope autoradiography. Int J Radiat Biol Relat Stud Phys Chem Med 41:633–643, 1982.
23. Itoh M, De Rooij DG, Jansen A, Drexhage HA. Phenotypical heterogeneity of testicular macrophages/dendritic cells in normal adult mice: an immunohistochemical study. J Reprod Immunol 28:217–232, 1995.
24. Yee JB, Hutson JC. Testicular macrophages: isolation, characterization and hormonal responsiveness. Biol Reprod 29:1319–1326, 1983.
25. Hutson JC, Garner CW, Doris PA. Purification and characterization of a lipophilic factor from testicular macrophages that stimulates testosterone production by Leydig cells. J Androl 17:502–508, 1996.
26. Wei RQ, Yee JB, Straus DC, Hutson JC. Bactericidal activity of testicular macrophages. Biol Reprod 38:830–835, 1988.
27. Hutson JC. Leydig cells do not have Fc receptors. J Androl 10:159–165, 1989.
28. Hutson JC, Stocco DM. Comparison of cellular and secreted proteins of macrophages from the testis and peritoneum on two-dimensional polyacrylamide gels: evidence of tissue specific function. Reg Immunol 2:249–253, 1989.
29. Yee JB, Hutson JC. Effects of testicular macrophage-conditioned medium on Leydig cells in culture. Endocrinology 116:2682–2684, 1985.
30. Nes WD, Lukyanenko YO, Jia ZH, Quideau S, Howald WN, Pratum TK, West RR, Hutson JC. Identification of the lipophilic factor produced by macrophages that stimulates steroidogenesis. Endocrinology 141:953–958, 2000.
31. Lukyanenko YO, Carpenter AM, Boone MM, Baker CR, McGunegle DE, Hutson JC. Specificity of a new lipid mediator produced by testicular and peritoneal macrophages on steroidogenesis. Int J Androl 23:258–265, 2000.
32. Stocco DM, Wang X, Jo Y, Manna PR. Multiple signaling pathways regulating steroidogenesis and StAR expression: more complicated than we thought. Mol Endocrinol 19:2647–2659, 2005.
33. Simpson ER. Cholesterol side-chain cleavage, cytochrome P450, and the control of steroidogenesis. Mol Cell Endocrinol 13:213–227, 1979.
34. Lund EG, Kerr TA, Sakai J, Li WP, Russell DW. cDNA cloning of mouse and human cholesterol 25-hydroxylases, polytopic membrane proteins that synthesize a potent oxysterol regulator of lipid metabolism. J Biol Chem 273:34316–34327, 1998.
35. Lukyanenko YO, Chen JJ, Hutson JC. Production of 25-hydroxycholesterol by testicular macrophages and its effects on Leydig cells. Biol Reprod 64:790–796, 2001.
36. Storey MK, Byers DM, Cook HW, Ridgway ND. Cholesterol regulates oxysterol binding protein (OSBP) phosphorylation and Golgi localization in Chinese hamster ovary cells: correlation with stimulation of sphingomyelin synthesis by 25-hydroxycholesterol. Biochem J 336(Pt 1):247–256, 1998.
37. Dushkin M, Schwartz Y, Volsky N, Musatov M, Vereschagin E, Ragino J, Perminova O, Kozlov V. Effects of oxysterols upon macrophage and lymphocyte functions in vitro. Prostaglandins Other Lipid Mediat 55:219–236, 1998.
38. Zhou Q, Jimi S, Smith TL, Kummerow FA. The effect of 25-hydroxycholesterol on accumulation of intracellular calcium. Cell Calcium 12:467–476, 1991.
39. Lizard G, Deckert V, Dubrez L, Moisant M, Gambert P, Lagrost L. Induction of apoptosis in endothelial cells treated with cholesterol oxides. Am J Pathol 148:1625–1638, 1996.
40. Wohlfeil ER, Campbell WB. 25-hydroxycholesterol enhances eicosanoid production in cultured bovine coronary artery endothelial cells by increasing prostaglandin G/H synthase-2. Biochim Biophys Acta 1345: 109–120, 1997.
41. Accad M, Farese RV Jr. Cholesterol homeostasis: a role for oxysterols. Curr Biol 8:R601–R604, 1998.
42. Olkkonen VM, Lehto M. Oxysterols and oxysterol binding proteins: role in lipid metabolism and atherosclerosis. Ann Med 36:562–572, 2004.
43. Schroepfer GJ Jr. Oxysterols: modulators of cholesterol metabolism and other processes. Physiol Rev 80:361–554, 2000.
44. Christenson LK, McAllister JM, Martin KO, Javitt NB, Osborne TF, Strauss JF III. Oxysterol regulation of steroidogenic acute regulatory protein gene expression: structural specificity and transcriptional and posttranscriptional actions. J Biol Chem 273:30729–30735, 1998.
45. King SR, Matassa AA, White EK, Walsh LP, Jo Y, Rao RM, Stocco DM, Reyland ME. Oxysterols regulate expression of the steroidogenic acute regulatory protein. J Mol Endocrinol 32:507–517, 2004.
46. Lukyanenko YO, Carpenter AM, Brigham DE, Stocco DM, Hutson JC. Regulation of Leydig cells through a steroidogenic acute regulatory protein-independent pathway by a lipophilic factor from macrophages. J Endocrinol 158:267–275, 1998.
47. Bergh A, Damber JE, van Rooijen N. Liposome-mediated macrophage depletion: an experimental approach to study the role of testicular macrophages in the rat. J Endocrinol 136:407–413, 1993.
48. Gaytan F, Bellido C, Morales C, Garcia M, van Rooijen N, Aguilar E. In vivo manipulation (depletion versus activation) of testicular macrophages: central and local effects. J Endocrinol 150:57–65, 1996.
49. Cohen PE, Chisholm O, Arceci RJ, Stanley ER, Pollard JW. Absence of colony-stimulating factor-1 in osteopetrotic (csfmop/csfmop) mice results in male fertility defects. Biol Reprod 55:310–317, 1996.
50. van Rooijen RN. Liposomes as an in vivo tool to study and manipulate macrophage function. Res Immunol 143:177–178, 1992.
51. van Rooijen RN. Liposome-mediated elimination of macrophages. Res Immunol 143:215–219, 1992.
52. Brigham DE, Little G, Lukyanenko YO, Hutson JC. Effects of clodronate-containing liposomes on testicular macrophages and Leydig cells in vitro. J Endocrinol 155:87–92, 1997.
53. Stanley ER, Berg KL, Einstein DB, Lee PS, Yeung YG. The biology and action of colony stimulating factor-1. Stem Cells 12(Suppl 1):15–24, 1994.
54. Cohen PE, Hardy MP, Pollard JW. Colony-stimulating factor-1 plays a major role in the development of reproductive function in male mice. Mol Endocrinol 11:1636–1650, 1997.
55. Cohen PE, Nishimura K, Zhu L, Pollard JW. Macrophages: important accessory cells for reproductive function. J Leukoc Biol 66:765–772, 1999.
56. Lukyanenko Y, Chen JJ, Hutson JC. Testosterone regulates 25-hydroxycholesterol production in testicular macrophages. Biol Reprod 67:1435–1438, 2002.
57. Chen JJ, Lukyanenko Y, Hutson JC. 25-hydroxycholesterol is produced

by testicular macrophages during the early postnatal period and influences differentiation of Leydig cells in vitro. Biol Reprod 66: 1336–1341, 2002.

58. Hutson JC. Changes in the concentration and size of testicular macrophages during development. Biol Reprod 43:885–890, 1990.
59. Hales DB. Testicular macrophage modulation of Leydig cell steroidogenesis. J Reprod Immunol 57:3–18, 2002.
60. Moore C, Hutson JC. Physiological relevance of tumor necrosis factor in mediating macrophage-Leydig cell interactions. Endocrinology 134: 63–69, 1994.
61. Bryniarski K, Szczepanik M, Ptak M, Ptak W. Modulation of testicular macrophage activity by collagenase. Folia Histochem Cytobiol 43:37–41, 2005.
62. Bryniarski K, Szczepanik M, Ptak M, Ptak W. The influence of collagenase treatment on the production of TNF-alpha, IL-6 and IL-10 by testicular macrophages. J Immunol Methods 301:186–189, 2005.
63. Bryniarski K, Szewczyk K, Ptak M, Bobek M, Ptak W. A two step procedure to fractionate mouse testicular macrophages with different cytokine profiles. Arch Immunol Ther Exp (Warsz) 50:225–229, 2002.
64. Bryniarski K, Szczepanik M, Maresz K, Ptak M, Ptak W. Subpopulations of mouse testicular macrophages and their immunoregulatory function. Am J Reprod Immunol 52:27–35, 2004.
65. Dirami G, Poulter LW, Cooke BA. Separation and characterization of Leydig cells and macrophages from rat testes. J Endocrinol 130:357–365, 1991.
66. O'Bryan MK, Gerdprasert O, Nikolic-Paterson DJ, Meinhardt A, Muir JA, Foulds LM, Phillips DJ, de Kretser DM, Hedger MP. Cytokine profiles in the testes of rats treated with lipopolysaccharide reveal localized suppression of inflammatory responses. Am J Physiol Regul Integr Comp Physiol 288:R1744–R1755, 2005.
67. Cohen PE, Pollard JW. Normal sexual function in male mice lacking a functional type I interleukin-1 (IL-1) receptor. Endocrinology 139:815–818, 1998.
68. Rothe J, Lesslauer W, Lotscher H, Lang Y, Koebel P, Kontgen F, Althage A, Zinkernagel R, Steinmetz M, Bluethmann H. Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature 364:798–802, 1993.
69. Peschon JJ, Torrance DS, Stocking KL, Glaccum MB, Otten C, Willis CR, Charrier K, Morrissey PJ, Ware CB, Mohler KM. TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. J Immunol 160:943–952, 1998.
70. Dalrymple SA, Lucian LA, Slattery R, McNeil T, Aud DM, Fuchino S, Lee F, Murray R. Interleukin-6-deficient mice are highly susceptible to Listeria monocytogenes infection: correlation with inefficient neutrophilia. Infect Immun 63:2262–2268, 1995.
71. Weissman BA, Niu E, Ge R, Sottas CM, Holmes M, Hutson JC, Hardy MP. Paracrine modulation of androgen synthesis in rat leydig cells by nitric oxide. J Androl 26:369–378, 2005.
72. Hales DB. Testicular macrophage modulation of Leydig cell steroidogenesis. J Reprod Immunol 57:3–18, 2002.
73. Hedger MP. Macrophages and the immune responsiveness of the testis. J Reprod Immunol 57:19–34, 2002.
74. Hedger MP. Macrophages and the immune responsiveness of the testis. J Reprod Immunol 57:19–34, 2002.
75. Lysiak JJ. The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. Reprod Biol Endocrinol 2:9, 2004.
76. Fortune JE, Armstrong DT. Androgen production by theca and granulosa isolated from proestrous rat follicles. Endocrinology 100: 1341–1347, 1977.
77. Dorrington JH, Moon YS, Armstrong DT. Estradiol-17beta biosynthesis in cultured granulosa cells from hypophysectomized immature rats: stimulation by follicle-stimulating hormone. Endocrinology 97: 1328–1331, 1975.
78. Resko JA, Roselli CE. Prenatal hormones organize sex differences of the neuroendocrine reproductive system: observations on guinea pigs and nonhuman primates. Cell Mol Neurobiol 17:627–648, 1997.
79. Payne AH, O'Shaughnessy PJ. Structure, function and regulation of steroidogenic enzymes in the Leydig cell. In: Payne AM, Hardy MP, Russell LD, Eds. The Leydig Cell. Vienna: Cache River Press, pp259–286, 1996.
80. Zhang YM, Rao C, Lei ZM. Macrophages in human reproductive tissues contain luteinizing hormone/chorionic gonadotropin receptors. Am J Reprod Immunol 49:93–100, 2003.
81. Aubry F, Habasque C, Satie AP, Jegou B, Samson M. Expression and regulation of the CC-chemokine monocyte chemoattractant protein-1 in rat testicular cells in primary culture. Biol Reprod 62:1427–1435, 2000.
82. Kato H, Horino A, Taneichi M, Fukuchi N, Eto Y, Ushijima H, Komuro K, Uchida T. Macrophage inhibition of lymphocyte and tumor cell growth is mediated by 25-hydroxycholesterol in the cell membrane. Int Arch Allergy Immunol 117:78–84, 1998.