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Number, distribution pattern, and identification of macrophages in the testes of infertile men

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Objective: To investigate the number, location, and secretory products of macrophages in human testes showing normal and abnormal spermatogenesis.

Design: Evaluation of testicular biopsies with the use of immunohistochemistry, laser capture microdissection, and reverse transcriptase polymerase chain reaction.

Setting: University research and clinical institutes.

Patient(s): Infertile men with germ cell arrest (n = 10), Sertoli cell only (n = 8), or mixed atrophy (n = 7) syndromes, and with cases of idiopathic infertility showing normal spermatogenesis (n = 8).

Intervention(s): Diagnostic testicular biopsy was performed on participants.

Main Outcome Measure(s): We recorded the location, number, distribution, and cytokine expression of human testicular macrophages.

Result(s): CD68-positive macrophages were found in the testes of all groups analyzed. These macrophages expressed the genes for interleukin 1 and tumor necrosis factor-α, and were located in the interstitium, tubular wall, and tubular lumen. In Sertoli cell only and germ cell arrest syndromes, the overall macrophage number was increased over twofold. In all pathologic states, there was a significant shift of these cells from the interstitium to the tubules.

Conclusion(s): Our study suggests that increased numbers of CD68-positive macrophages directly (via phagocytosis) or indirectly (via paracrine actions exerted through their secretory products) are involved in the regulation of steroidogenesis, Sertoli cell activity, germ cell survival, and, in consequence, in the pathogenesis or maintenance of infertility states in the human testes. (Fertil Steril® 2002;78:298–306. ©2002 by American Society for Reproductive Medicine.)

Key Words: Testis, macrophages, human, infertility, CD68, IL1, TNFα

Leukocytes and macrophages are found within the testes of most, if not all, mammals (1–4). Circulating monocytes reach the gonad during fetal life and are a major source of testicular macrophages (5, 6), but the number of macrophages and their functions are largely determined by the local environment (7, 8). Thus, recent findings indicate that macrophages are able to undergo proliferation inside the testis during sexual development, as well as in adulthood, in certain cases (6, 9).

Concerning human testis, early morphological studies described the presence of macrophages in the interstitium and occasionally in the lamina propria of seminiferous tubules (4, 10).

The biological significance of these cells for testicular function is unknown, but they appear to be involved in immunologic surveillance, immunoregulation, and tissue remodeling. The phagocytic capacity of testicular macrophages became evident after the administration of the specific Leydig cell cytotoxin, ethylene dimethane sulfonate (EDS) (11). Macrophages show an astonishing ability to secrete biologically active molecules. More than 100 macrophage secretory products have been identified (12–14). Secretory capacity of macrophages implies a potential role of testicular macro-

phages in paracrine interactions with other gonadal cell populations. Several studies have suggested that testicular macrophage products such as cytokines and 25-hydroxycholesterol have specific effects on Leydig cell development and steroidogenesis, as well as on Sertoli cells and spermatogenic cells (8, 15–17). In spite of these results, it is not known what roles macrophages play in spermatogenic defects resulting in male infertility.

Male infertility can result from congenital, infective, vascular, immunologic, and autoimmune reasons. In approximately 40% to 50% of cases, the cause is as yet unknown (18).

In an attempt to identify cellular factors involved in local regulation of testicular function and their possible role in the pathophysiology of infertility, we have previously examined mast cells in testicular biopsies of men with normal and abnormal spermatogenesis (19). In the present study, we used the same human biopsies (i.e., we used biopsies revealing normal spermatogenesis and no obvious alteration as the normal group; the abnormal groups consisted of biopsies revealing Sertoli cell only syndrome, as well as germ cell arrest syndrome). We also used a new set of specimens classified as biopsies with mixed atrophy (MA) syndrome. "Mixed atrophy" of seminiferous tubules represents a testicular disorder characterized by tubules with various degrees of degeneration of the germinal epithelium, ranging from full spermatogenesis up to Sertoli cell only (SCO) and seminiferous tubule fibrosis within the same biopsy (20). Using these samples we explored whether testicular macrophages and their secretory products are involved in pathogenesis and/or maintenance of impaired spermatogenesis.

Macrophage morphology, number, and location as well as the expression of macrophage secretory products in those biopsies were evaluated employing immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR) analysis from paraffin-embedded material scratched from the slides or collected by laser capture microdissection (LCM) and laser pressure catapulting (LPC) of immunostained cells.

MATERIALS AND METHODS

Human Biopsies

Archival testicular biopsies from adult men with fertility problems (age range, 28 to 37 years) were assigned to the following groups: specimens from men with cases of idiopathic infertility revealing normal spermatogenesis without obvious alterations (n = 7), specimens from patients with SCO syndrome (n = 7), and specimens from patients with severe hypospermatogenesis and germ cell arrest (GA) syndrome (n = 9). Follicle-stimulating hormone (FSH) values of the three groups were determined and averaged (mean ± SEM) as follows: normals 6.6 ± 1.3 U/L, GA syndrome 13.2 ± 3.3 U/L, and SCO syndrome 21.1 ± 7.0 U/L (normal range, 1–14 U/L). All of those biopsies were used in a previous study to examine mast cells in human testes (19).

In the present work, we also analyzed a new group of specimens from patients with MA syndrome (n = 6). The FSH values in MA group were mean ± SEM: 23.2 ± 7.2 U/L. Three specimens showed levels on the borderline of the normal range, and other three samples presented values higher than 30 U/L.

The specimens were fixed in Bouin's fixative, embedded in paraffin, and processed for histologic studies, immunohistochemical studies, and for LCM and LPC techniques. The evaluation of human specimens was approved by the local ethics committee of the university.

Histologic and Immunohistochemical Assays

Human testicular biopsies were examined by immunohistochemistry as described previously (19, 21).

Macrophages were determined using commercially available monoclonal antibodies (mouse anti-human CD68, DAKO, clone PG-M1, Hamburg, Germany, 1:100; mouse anti-human CD163, DPC Biermann GmbH, Bad Nauheim, Germany, 1:100). Human testicular biopsies were routinely pretreated with proteinase K (10 μg/mL in phosphate-buffered saline, PBS) for 2 minutes at room temperature before incubation with the first antibody.

For control purposes, the first antiserum was omitted, or incubation with normal mouse sera instead was carried out. Sections were examined with a Zeiss Axiovert (Oberkochen, Germany) microscope.

Mast cells were identified after staining with 0.3% Alcian blue in acetic acid pH 1.5 and were subsequently examined in a light microscope.

RT-PCR Analysis

Gene expression in fixed and paraffin-embedded sections of human biopsies was performed as described (22). In brief, deparaffinized 5 μm thick consecutive sections of biopsies, in which we found CD68-immunoreactive cells, were assessed after scratching them from the glass slides. The RNA was extracted using the Purescript kit (Biozym, Hessisch Oldenburg, Germany), and RT was performed as described elsewhere (22).

The first set of oligonucleotide primers used to amplify a human CD68 cDNA consisted of an 18-mer sense primer 5′-CCATGAGGCTGGCTGTGC and an 18-mer antisense primer 5′-GCAGGACTGTGAGTGGCA complementary to the human CD68 mRNA (Genebank accession number: NM_001251). The PCR amplification (Taq-polymerase; Promega, Mannheim, Germany) consisted of 35 cycles of denaturing (94°C, 1 minute), annealing (60°C, 1 minute), and extension (72°C, 1 minute). The length of the expected cDNA was 337 base pairs (bp). This reaction product was purified with a spin column (MinElute PCR purification kit Qiagen, Hilden, Germany) to remove primers, nucleotides,

TABLE 1

Primers (first and second nested set) for PCR amplification of cDNAs obtained after RT using RNA from paraffin-embedded material collected by laser capture microdissection (LCM) and laser pressure catapulting (LPC) of immunostained cells.

Target	Primers	Sense primers	Antisense primers	Expected cDNA length (bp)
CD163	First set	5′-TGTAGCGGGAGAGTGGAA	5′-CTGAGCAGGTCACTCCAG	280
	Second nested set	5′-TAATGGCTGGAGCATGGA	5′-CAAAGAGCTGACTCATTC	153
IL1α	First set	5′-CTGTCTCTGAATCAGAAATCC	5′-GTCAAATTTCACTGCTTCATC	420
	Second nested set	5′-GCTGCATGGATCAATCTG	5′-TCACATTGCTCAGGAAGC	231
IL1β	First set	5′-CAGGCTGCTCTGGGATTC	5′-GCCTCGTTATCCCATGTG	393
	Second nested set	5′-CCTAAACAGATGAAGTGC	5′-AGCATCTTCCTCAGCTTG	149
IL6	First set	5′-CTCCTTCTCCACAAGCGC	5′-TTGCCGAAGAGCCCTCAG	628
	Second nested set	5′-AGCCACTCACCTCTTCAG	5′-CTGGAGGTACTCTAGGTA	257
TNFα	First set	5′-GGCAGCTCCAGTGGCTGA	5′-ATGATCCCAAAGTAGACC	394
	Second nested set	5′-ACACCATCAGCCGCATCG	5′-GATCTCAGCGCTGAGTCG	176
Tryptase	First set	5′-TGCTGGAGCTGGAGGAGC	5′-AGGTGCCATTCACCTTGC	336
	Second nested set	5′-GTGCTGGGTCACTGGCTG	5′-CCCGCACACAGCATGTC	177
Chymase	First set	5′-AGGAGAAAGCCAGCCTGA	5′-ATGCAGATTTTGTCTTCC	246
	Second nested set	5′-CTGGCTGTGGGGACACTC	5′-ATTGCCCACACACAGCTG	176
StAR	First set	5′-TGGAGAGGCTCTATGAAGAGC	5′-GCCACGTAAGTTTGGTCTTAG	336
	Second nested set	5′-GAGTGGAACCCCAATGTC	5′-GCACCATGCAAGTGGGAC	244

GenBank accession number for CD163: NM_004244, IL1α: NM_000575, IL1β: NM_000576, IL6: NM_000600, TNFα: NM_000594, Tryptase: NM_012217, Chymase: XM_007317, StAR: NM_000349.

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and small cDNA fragments, and an aliquot was used for a second PCR with nested oligonucleotide primers. These primers were complementary to nucleotides 5′-TGCTGGGGCTACTGGCAG and to nucleotides 5′-CTGGTGGCAGTGCTATTG in human CD68 mRNA. The length of the expected cDNA was 271 bp. The PCR reaction products were separated on 2% agarose gels and visualized with ethidium bromide.

To further identify the cDNAs, we sequenced them using a fluorescence-based dideoxy-sequencing reaction. An automated sequence analysis was performed on an ABI model 373A DNA sequencer.

Laser Capture Microdissection and Laser Pressure Catapulting Techniques

Human biopsies embedded in paraffin were cut in sections (5 μm) and mounted onto a 1.35 μm thin polyethylene naphtalene membrane pasted to a glass slide that was pretreated with UV light for 30 minutes. The sections were deparaffinized, immunostained with anti-CD68 antiserum (DAKO) as described above, and processed for hematoxylin staining.

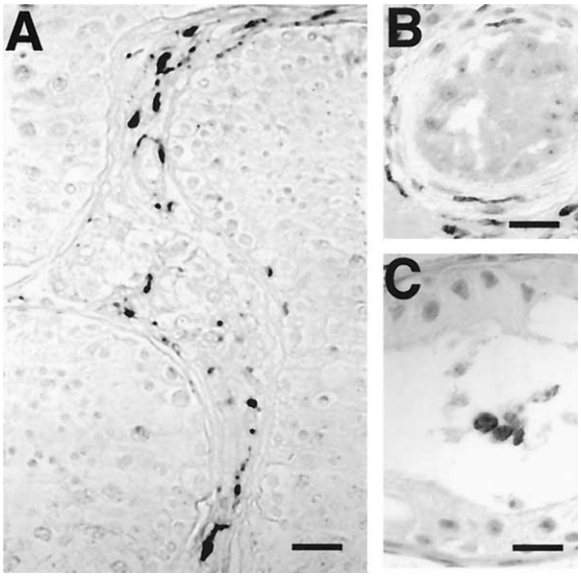
Laser capture microdissection (LCM) and laser pressure catapulting (LPC) were performed as described elsewhere (23). In brief, we employed the high photonic energy of the focused nitrogen laser of the Robot-MicroBeam (P.A.L.M. GmbH Mikrolaser Technologie, Bernried, Germany); CD68-immunoreactive cells, mainly those located in the interstitium and in the tubular wall, were precisely circumscribed to isolate them from the surrounding tissue. Circumscription of the target specimen resulted in a gap, free of any biological material, which separated the target sample from the surrounding material. The width of the gap was between 1 and 10 μm, depending on the objective and on the absorption behavior of the specimen.

Following the LCM, the laser was focused below the microdissected target specimen and the energy was increased by 40% in comparison to the level used for microdissection. With a single laser shot the microdissected samples were ejected from the object slide and catapulted directly into the cap of a microfuge tube. We added 50 μL of RNA stabilization reagent (RNEasy Protect Mini-kit, Qiagen) into the cap. Finally, the samples were spun down into the tube and frozen at −70°C until RT-PCR assays were performed. To test specificity, for the consecutive section we eliminated CD68-positive cells by a few directed laser shots; the area was circumscribed, and the remaining material was catapulted into the cap of a microfuge tube and used as negative control.

For all other genes of interest, RT as well as first PCR and second PCR amplification, using nested primers, were performed as described above for CD68. Oligonucleotide primers employed and cDNAs isolated are summarized in Table 1. When information about exon structure was available in Genebank, nested oligonucleotide primers were designed to be homologous to different exons (CD68, tryptase, StAR, and interleukin [IL] 1β genes).

FIGURE 1

CD68-immunoreactive macrophages in the human testis. (**A**), Immunoreactive macrophages in a testis with normal spermatogenesis are seen in the interstitial compartment (bar equivalent to 10 μm). (**B**), Several peritubular mast cells are seen in the testis of a patient with Sertoli cell–only syndrome (bar equivalent to 20 μm). (**C**), CD68-positive macrophages are seen in the tubular lumen of a patient with germ cell arrest syndrome (bar equivalent to 30 μm).



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The identity of the PCR products was verified by sequencing as was described for CD68.

Quantification of CD68-Immunoreactive Macrophages

The number of CD68-immunoreactive structures (cells larger than 3 μm in which both nucleus and cell bodies were clearly identified) was quantified with a Zeiss microscope (Jena, Germany) with a magnification of $\times$400 and a gridded eyepiece as previously described (19). In each testicular section, six to eight fields were evaluated for the presence of total macrophages, interstitial macrophages, macrophages in the tubular wall, and macrophages in the tubular lumen; and the seminiferous tubules sectioned were counted.

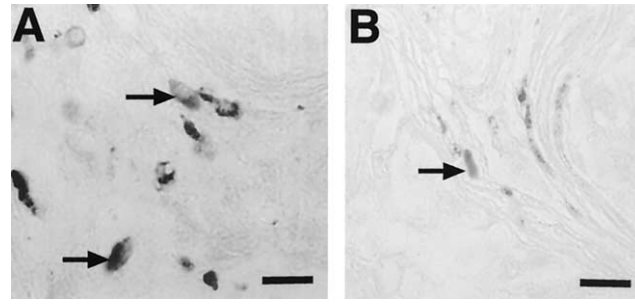
The results were expressed as immunoreactive CD68 macrophages/tubule and immunoreactive CD68 macrophages/mm^2 as previously described (19).

Statistical Analysis

The results were analyzed statistically with a computer program (Prism; GraphPad Software, Inc., San Diego, CA) and by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparisons. Data are expressed as mean + standard error of the mean (SEM).

FIGURE 2

Anatomical proximity between CD68-positive macrophages and mast cells. (**A**), CD68-immunoreactive macrophages (avidin-biotin peroxidase method) and Alcian blue-positive mast cells (*arrows*) in the interstitial space of a testis with normal spermatogenesis (bar equivalent to 15 μm). (**B**), Several CD68-immunoreactive macrophages (avidin-biotin peroxidase method) and an Alcian blue-positive mast cell (*arrow*) in the wall of seminiferous tubules of a testis with Sertoli cell–only syndrome (bar equivalent to 15 μm).



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RESULTS

CD68-Positive Cells in Normal and Pathologic Human Biopsies

The lysosome marker CD68 identified macrophages in all the specimens analyzed. Macrophages were mainly localized in the interstitium, but were also found in the wall and inside the lumen of seminiferous tubules (Fig. 1A–C).

A double-staining method allowed us to clearly identify close anatomical proximity between mast cells (Alcian blue staining) and macrophages (CD68-positive, avidin-biotin peroxidase method) in the interstitium and in the wall of seminiferous tubules (Fig. 2A, B). We did not observe colocation between CD68-immunoreactive cells and Alcian blue–positive cells, indicating that CD68 antisera did not cross-react with mast cell antigens (see Fig. 2A, B).

Results from the RT-PCR analysis and sequencing, with RNA extracted from sections of biopsy material, indicated furthermore that CD68 gene expression is detectable in normal, GA, SCO, and MA syndrome testes (Fig. 3).

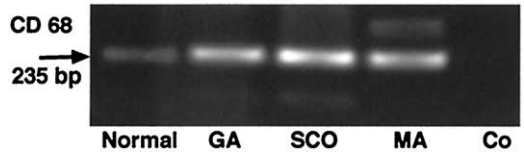
Some, but not all, CD68-immunoreactive macrophages found in the human testis were also positively stained for CD163, a glycoprotein member of the scavenger receptor family (Fig. 4A, B).

Evidence for Gene Expression of Macrophage Secretory Products in CD68-Positive Cells

The CD68-immunoreactive macrophages from normal and SCO, GA, and MA biopsies (n = 15 to 20 cells per biopsy) were isolated employing LCM and LPC techniques (Fig. 5A, B). The RT-PCR analysis of these cells followed

FIGURE 3

Identification of CD68 gene expression in human testis with RT-PCR analysis of RNA isolated from paraffin-embedded human testicular biopsies. After sequencing fragments were shown to correspond to human CD68, ethidium bromide-stained agarose gel shows a 235-bp cDNA fragment after a second nested PCR. (GA: germ arrest syndrome; SCO: Sertoli cell–only syndrome; MA: mixed atrophy syndrome; Co: no input cDNA for PCR amplification.)



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by sequencing indicated that CD68-immunoreactive testicular macrophages express CD68, CD163, IL1α, IL1β, and tumor necrosis factor-α (TNFα) genes (Fig. 6). Nevertheless, expression of IL6 was not detected (see Fig. 6).

Expression of tryptase (mast cell marker), chymase (mast cell marker), and StAR (Leydig cell marker) in these cells was not found, indicating that the material employed in LCM and LPC was not contaminated with other testicular cell populations (see Fig. 6).

Confirming the specificity of LCM and LPC techniques in the material obtained from sections in which the CD68-positive cells were eliminated by a few directed laser shots (see Fig. 5C, D), the expression of CD68, CD163, IL1α, IL1β, and TNFα genes was not detected (data not shown).

Quantification of CD68-Positive Macrophages

The numbers of CD68-immunoreactive macrophages (expressed per tubule and per mm^2) were increased in the biopsies with GA syndrome and SCO syndrome compared with normal biopsies without morphologic changes (Fig. 7). In GA syndrome and in SCO syndrome, the number of total testicular macrophages was doubled compared to normal sections. In MA biopsies, the number of total macrophages remained unchanged. In SCO syndrome and in GA syndrome, the number of peritubular macrophages was more than three times higher than in normal sections. In GA syndrome and MA syndrome, the number of intratubular macrophages was more than six times higher than found in normal biopsies (see Fig. 7).

FIGURE 4

CD68- and CD163-immunoreactive macrophages in the human testis. (**A**), Several CD68-positive macrophages (*arrows*) were also immunoreactive for (**B**) CD-163 antigen (*arrows*). Shown are consecutive sections of a Sertoli cell–only biopsy (bar equivalent to 15 μm).

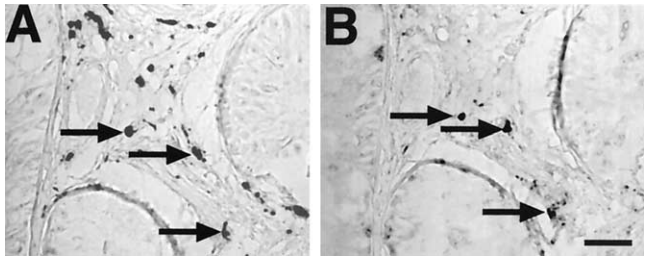

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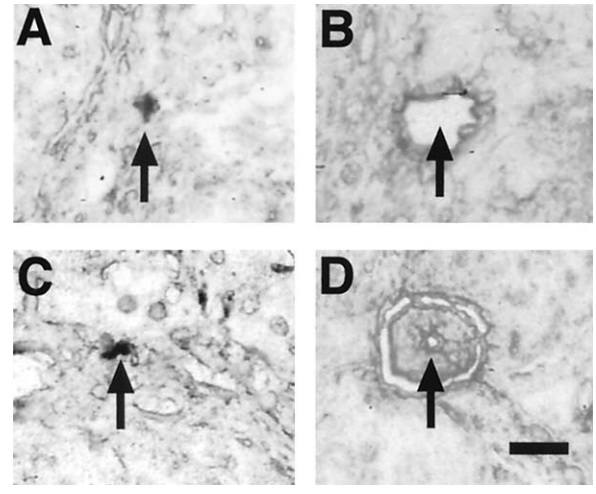
FIGURE 5

Laser capture microdissection (LCM) and laser pressure catapulting (LPC) of CD68-immunoreactive cells in human testicular biopsies. (**A**), A CD68 immunoreactive macrophage (*arrow*) is shown. (**B**), The same specimen is seen after microdissection of this CD68-immunoreactive cell (*arrow*) which was used for RT-PCR analysis (testis of a patient with Sertoli cell–only syndrome). (**C**), As in *A*, a CD68-positive cell (*arrow*) is shown before microdissection. (**D**), In this case, however, this cell was destroyed by a few laser shots (*arrow*) and the remaining material was catapulted into the cap of a microfuge tube to be used as negative control (testis of a patient with germ arrest syndrome). (Bar equivalent to 30 μm.)



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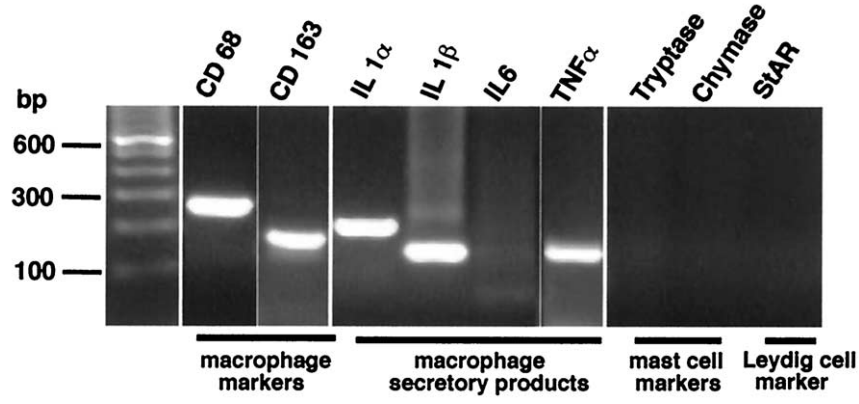
DISCUSSION

The salient results of this study are that [1] CD68 and CD163 immunoreactive macrophages are present in human testes and express the inflammatory cytokines IL1 (α and β isoforms) and TNFα, and [2] the number of testicular macrophages, particularly those located in the lumen of the seminiferous tubules, is significantly increased in testis with hypospermatogenesis or complete absence of germ cells,

FIGURE 6

Gene expression analysis of macrophage secretory products by laser capture microdissection (LCM), laser pressure catapulting (LPC), and RT-PCR of CD68-positive cells from human testis. Results of RT-PCR analysis of RNA isolated by LCM and LPC of CD68-positive macrophages (n = 15 to 20 cells per biopsy) from human testicular biopsies showing normal spermatogenesis. Ethidium bromide-stained agarose gel shows cDNA fragments after a second nested PCR, which were shown by sequencing to be identical to the published sequences. Comparable results were obtained when Sertoli cell–only, mixed atrophy, and germ arrest biopsies were employed.



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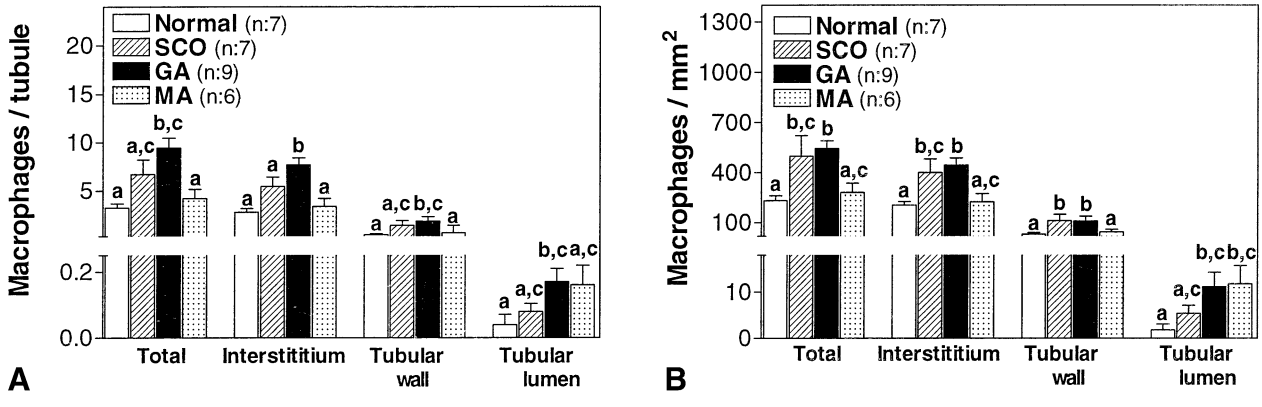
suggesting that macrophages and/or their secretory products may be involved in the cause of male infertility.

This is the first report showing existence of CD68- and CD163-positive macrophages in the human testis. Until now, resident macrophages in mammalian testes were characterized by their phagocytic activity and their morphologic features such as lysosomal inclusions and distinct heterochromatin, or they were identified by other markers, such CD4+, anti-Leu M3, HLA-ABC, or HLA-DR (4, 24–26). These markers are known to be not exclusive for macrophages and may be present on other cells, including endothelial cells and lymphocytes.

The monoclonal antibody used for our study in the human species, CD68, is a 110-kd glycosylated type 1 transmem-

FIGURE 7

Quantification of CD68-immunostained macrophages. Quantitative results are expressed as (**A**) macrophages per tubule and (**B**) macrophages per mm^2 in normal, Sertoli cell–only syndrome (SCO), germ arrest syndrome (GA), and mixed atrophy syndrome (MA) biopsies. The data represent mean + SEMs (n = 6 to 9). Identical letters indicate no difference between them; different letters indicate statistically significant differences ($P<.05$, Student-Newman-Keuls test).



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brane protein mainly located in lysosomes of macrophages. CD68 was found to be a highly specific marker for human macrophages. Its specificity to identify macrophages without cross-reactivity with other cells—including granulocytes, myeloid, lymphoid, epithelial, fibroblastic, myocytic, melanocytic, and germ cells—was previously studied by Falini et al. (27). In addition, the expression of the gene for CD68 was shown by a highly sensitive RT-PCR approach performed with mRNA isolated from consecutive sections of those sections, which contained CD68-immunoreactive cells.

We also employed the macrophage marker CD163 to further characterize these cells in the human testis. CD163 antigen is a glycoprotein member of the cysteine-rich scavenger receptor family located on human monocytes/macrophages and is expressed in the intermediate and late phase of inflammation (28). Some, but not all, of the CD68-immunoreactive macrophages found in the human testis also stained for CD163. These discrepancies may be associated with a different origin, degree of maturation, and/or activation of the testicular macrophage population.

In this study, we described a close anatomic proximity between macrophages and mast cells in the interstitial compartment and in the wall of seminiferous tubules. Functional interactions between mast cells and macrophages have been previously described (29–31). Interactions between mast cells and macrophages lead to proliferation of fibroblasts and collagen formation (32, 33). It is possible that as a consequence this may lead to fibrotic changes that we previously described in the tubular walls of patients with GA and SCO syndromes (19).

To identify the macrophage secretory products in the human testis, we employed LCM, LPC, and RT-PCR techniques. With LCM and LPC we could isolate CD68-immunoreactive macrophages, mainly those located in the interstitium and in the tubular wall. By RT-PCR we determined that CD68-immunoreactive cells express the genes for CD68 and CD163, but they also express genes for specific macrophages markers including IL1α, IL1β, and TNFα. Mast cell product mRNAs (tryptase, chymase) and mRNA of the Leydig cell activity marker (StAR) were not detected. These results indicate that the material obtained by LCM and LPC was not contaminated with other testicular cell populations; consequently, the expression of IL1α, IL1β, and TNFα genes can be related to testicular macrophages.

Three well-known macrophage cytokines—TNFα, IL1α, and IL1β—have also been reported to be secreted from rat testicular macrophages (14, 34, 35). The role of these cytokines in the human testis is not known. Macrophages are often closely associated with Leydig cells, and highly specialized cell–cell cytoplasmatic contacts develop early in adult life between the two cell types (36, 37). Several reports indicate a modulatory effect of IL1 and TNFα on steroidogenesis (38–44). The available reports are somewhat contradictory, showing both inhibitory and stimulatory effects of IL1 and TNFα on androgen synthesis depending on the experimental conditions. Khan et al. (45) described an important role that IL1 plays in proliferation of Leydig cells.

Also, TNFα enhances expression of growth factors in Sertoli cells and production of metabolites important for germ cells (46), and IL1 regulates nitric oxide production, gamma-glutamyl transpeptidase activity, and lactate generation in Sertoli cells (47–49). In addition to the modulatory action exerted by macrophages and their secretory products on steroidogenesis and Sertoli cell activity, testicular macrophages secrete proinflammatory as well as anti-inflammatory cytokines and exert an important trophic and/or scavenger role in tissue morphogenesis and function (50, 51).

Our results show that the number of macrophages is increased in testes of men showing impaired spermatogenesis. The retrospective evaluation of random human testicular biopsies does not allow us to perform a more detailed quantification. Taking into account that the diameter of the seminiferous tubules changes in different pathological states, the results were not only expressed as CD68-positive macrophages per mm^2 but also as CD68-positive macrophages per seminiferous tubules; for this we used the approach previously described by Nagai et al. (52) and Meineke et al. (19) for evaluation of mast cell populations in human testicular biopsies.

The increased number of testicular macrophages detected in pathological states was accompanied by a significant elevation of serum FSH levels. Whether macrophages and FSH in the human are interrelated is not clear. Although several reports show that testicular macrophages possess FSH receptors (53, 54) that could influence activity and proliferation of gonadal macrophages (55–58), Carpenter et al. (59) did not detect FSH receptor mRNA in testicular macrophages isolated from adult rats.

In addition to the previously reported interstitial macrophages (4, 25, 60), we also found macrophages in the limiting membrane of seminiferous tubules and in the tubular lumen of the human testis, mainly in pathological states. In accordance with these results, in regressed testes of a seasonal animal, the swan, an invasion of macrophages into the seminiferous tubules was reported (61). Moreover, degenerated tubules after long-term vasectomy in the guinea pig present macrophages impinging directly on the basement membrane (62). Testes from the homozygous symplastic spermatids mouse, which are infertile due to an abnormality in spermatogenesis leading to azoospermia, also contain intratubular macrophages (63). In humans, only few early studies described the presence of peritubular and intratubular macrophages in a carcinoma in situ of the testis (64) and in viral/bacterial infectious diseases with testicular lesions (26, 65).

Thus, our observation of the increased number of peritubular and intratubular macrophages found in SCO, GA, and

MA syndromes suggests a spermiophage role of macrophages in testes of infertile men with these syndromes.

In summary, although the mechanisms governing the changes detected in the number and distribution of CD68-macrophages in testes with abnormal spermatogenesis are currently unexplored, our results imply that testicular macrophages are directly (via phagocytosis) or indirectly (via paracrine actions exerted through their secretory products) involved in the regulation of steroidogenesis, Sertoli cell activity, and/or germ cell survival and, in consequence, in the pathogenesis or maintenance of states of infertility in the human testes.

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