

# Phenotypic characterization of the immune and mast cell infiltrates in the human testis shows normal and abnormal spermatogenesis

Mahmoud R. Hussein, M.D., Ph.D.,<sup>a</sup> Eman S. Abou-Deif, M.D.,<sup>b</sup> Mohamed A. Bedaiwy, M.D.,<sup>c</sup> Tamer M. Said, M.D.,<sup>d</sup> Mohammad G. Mustafa, M.D.,<sup>a</sup> Essam Nada, M.D.,<sup>c</sup> Alae Ezat, M.D.,<sup>f</sup> and Ashok Agarwal, Ph.D., H.C.L.D.<sup>d</sup>

<sup>a</sup>Departments of Pathology, <sup>b</sup>Histology, <sup>c</sup>Gynecology and Obstetrics, <sup>e</sup>Dermatology, and <sup>f</sup>Urology, Faculties of Medicine, Assuit and South Valley Universities, Assiut, Egypt; and <sup>d</sup>Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute, Department of Obstetrics and Gynecology, The Cleveland Clinic Foundation, Cleveland, Ohio

**Objective:** To assess the types, distributions, and numbers of immune cell infiltrates in the testes of men with azoospermia.

**Design:** Prospective study.

**Setting:** University research and clinical institutes.

**Patient(s):** Thirty-one men with azoospermia showing normal spermatogenesis ( $n = 10$ ), germ cell maturation arrest (GA,  $n = 12$ ), and Sertoli cell-only syndrome (SCO,  $n = 9$ ).

**Intervention(s):** Testicular tissue biopsies. Sections were stained with routine (hematoxyline and eosin), special (Masson Trichrome, Gordon, Periodic Acid Schiff, Aldehyde Fuchsin, and Orcein stains), and immunoperoxidase stains (using monoclonal antibodies for B and T cells and CD68 macrophages).

**Main Outcome Measure(s):** Serum levels of T, FSH, and LH in addition to histopathological analysis.

**Result(s):** Hormonal profiles were unremarkable in all patients. Marked deposition of the reticular and collagen fibers was seen more in abnormal than normal spermatogenesis. The immune (B and T lymphocytes and CD68 macrophages) and mast cells were found in the interstitium, tubular walls, and lumens of all the testes analyzed. The differential counts of these cells (B and T lymphocytes, CD68 macrophages, and mast cells, respectively) were higher in SCO ( $1.66 \pm 0.46$ ,  $9.14 \pm 1.30$ ,  $2.26 \pm 1.68$ ,  $3.35 \pm 0.23$ ) and GA ( $2.03 \pm 0.48$ ,  $4.70 \pm 1.00$ ,  $2.61 \pm 0.70$ , and  $4.18 \pm 0.13$ ) when compared with those in normal spermatogenesis ( $1.22 \pm 0.19$ ,  $5.41 \pm 0.58$ ,  $1.55 \pm 0.33$ , and  $2.26 \pm 0.13$ ). Increased cellular counts were not statistically significant for T and B cells and macrophages. However, these differences were statistically significant for mast cells.

**Conclusion(s):** Abnormal spermatogenesis is associated with increased numbers of the immune and mast cells. Our findings may reflect an exaggerated immune response in these cases. (Fertil Steril® 2005;83:1447–53. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** Lymphocytes, macrophages, mast cell, spermatogenesis

Spermatogenesis is under the control of a variety of factors, which include the immune system. Immune responses within the testis are regulated in a manner that provides protection for the developing male germ cells while permitting qualitatively normal inflammatory responses and protection against infection (1). These mechanisms are orchestrated in delicate ways that not only offer cytoprotection for the germ cells but also guard against autoimmune response. These mechanisms largely depend on [1] the local regulation of the immune cells and [2] clonal deletion, clonal anergy, and autoantigen segregation (2–4).

Monocytes and lymphocytes, which include both B and T (predominant) cells, are found in the testes of most mammals (4). They reach the gonads during fetal life and represent the source for resident macrophages. Their number and functions are determined by the local tissue factors and are involved in immune surveillance as well as immunoregulation. They characteristically have attenuated proinflammatory phenotype and release several molecules critical for the regulation of T and inhibin such as tumor necrosis factor and its receptor (5, 6).

In mammalian testis, mast cell mediators are involved in the regulation of steroidogenesis by Leydig cells (7). Mast cells contain protease tryptase, which acts as a potent mitogen for fibroblasts (8), and can enhance the synthesis of collagen (9) with subsequent fibrosis and thickening of the tubular wall, a common finding in infertility. Although the phenotypic characteristics of the testicular immune cells remain unknown, it is likely that they are affected by the

Received July 9, 2004; revised and accepted November 19, 2004.

Reprint requests: Dr. Ashok Agarwal, Professor of Surgery and Director, Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute, Department of Obstetrics-Gynecology, The Cleveland Clinic Foundation, 9500 Euclid Ave., Desk A19.1, Cleveland, Ohio 44195 (FAX: 216-445-6049; E-mail: Agarwaa@ccf.org).

status of the testicular somatic and germ cells (1). Therefore, it is conceivable that a possible relation exists among the immune cells, mast cells, and abnormal spermatogenesis. In this study, we hypothesized that abnormal spermatogenesis is associated with alterations in the characteristics of the testicular immune and mast cells. To test our hypothesis, we carried out the current investigation. To achieve our goals, we examined the clinicopathological and immunologic features of testicular biopsies obtained from 31 azoospermic infertile men.

## MATERIALS AND METHODS

### Patients

The research protocol was approved by the Ethics Committee of the Faculty of Medicine, Assiut University, Egypt. All patients provided written consent. Our study included 31 men with azoospermia with primary infertility who were admitted to the Urology Department, Assiut University Hospitals, during the period from December 2002 to December 2003. Their ages ranged from 25 to 35 years. The diagnosis was based on clinical examination, semen analysis, and hormonal profile (FSH, LH, and T).

### Testicular Biopsies

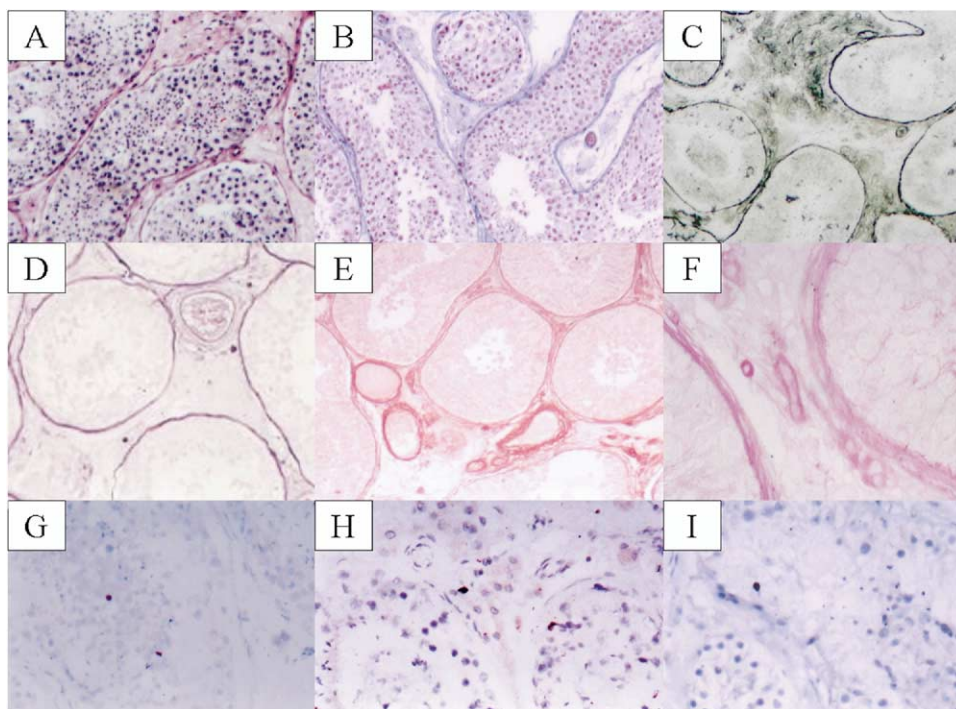
Open testicular biopsies were obtained under local anesthesia. Briefly, an incision was made in the tunica albuginea, pressure was exerted on the testis, and a small amount of testis (up to 5 mm) herniating through the incision was removed, dropped into Bouin's solution, and processed to paraffin blocks according to the standard procedure. Eight 4- $\mu$ m sections were cut from these blocks and stained with hematoxyline and eosin for routine histology and with special stains, which included Periodic Acid Schiff (glycogen), Masson Trichrome (collagen), Orcein (elastic stain), and Aldehyde Fuchsin (mast cells).

### Immunohistochemical Analysis

Immunostaining was carried out following other groups (10). Briefly, sections mounted on glass slides were deparaffinized and rehydrated through graded alcohols to water. Endogenous peroxidase activity was blocked with 0.6%  $H_2O_2$  in methanol. Sections were then immersed in the retrieval solution (10-mM sodium citrate buffer, pH 6.0) and subjected to enzymatic digestion (0.1% trypsin) for 20 minutes. Non-specific protein binding was blocked with 10-minute exposure to 10% normal goat serum. Sections were then incu-

## FIGURE 1

Morphological changes in complete spermatogenesis. (A) hematoxyline and eosin stain (10 $\times$ ); (B) Masson Trichrome stain (10 $\times$ ); (C) silver stain (10 $\times$ ); (D) Orcein stain (10 $\times$ ); (E) Periodic Acid Schiff's stain (10 $\times$ ); (F) Aldehyde Fuchsin stain (40 $\times$ ); (G) CD68 (macrophages) (40 $\times$ ); (H) CD20 (B lymphocytes, 40 $\times$ ); and (I) CD3 (T lymphocytes, 40 $\times$ ).



Hussein. Immune and mast cells in abnormal spermatogenesis. *Fertil Steril* 2005.

bated with mouse monoclonal antibodies for 30 minutes at room temperature (Clone PC3/188 A, Clone 124, and Clone M 0814 for CD3, CD20, and CD68, respectively). After brief rinsing in phosphate buffer solution, a catalyzed signal amplification system (K1500 from DAKO Corp., Carpinteria, CA) was used according to the manufacturer's instructions. Sections were next treated with peroxidase-labeled streptavidin for 30 minutes at room temperature and incubated with 1,4-diaminobenzidine and 0.06% H<sub>2</sub>O<sub>2</sub> for 5 minutes. They were counterstained with hematoxylin, dehydrated, cleared, and mounted under coverslips.

Known B and T cell lymphomas were used as positive controls for CD20 and CD3 staining. Alternatively, sinus histiocytosis was used as a positive control for CD68 macrophages. Additional sections of the testes as well as the positive controls were similarly stained, but with omission of the primary antibody to serve as negative controls (11).

### Statistical Analysis

One-way analysis of variance was used to detect the significance of differences between groups. Statistical analysis was conducted using Statistix software for Windows (Analytical Software, Tallahassee, FL).  $P < .01$  was considered statistically significant.

## RESULTS

### Clinical and Hormonal Features

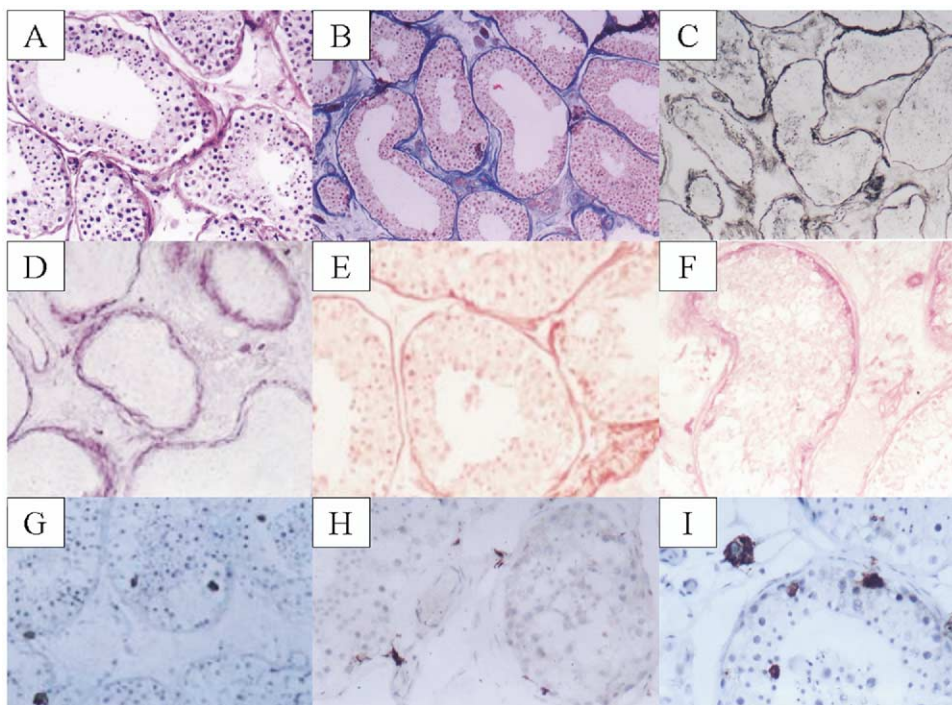
The age of the patients ranged from 25 to 35 years, and all had unremarkable clinical history and physical examination except for primary infertility. In all patients, serum levels of T, FSH, and LH were within normal ranges and semen analysis revealed azoospermia.

### Histological Features

The study period ran from December 2002 through December 2003. The pathological interpretations were carried out independently and reviewed by two independent authors (M.R.H. and E.S.A.) without prior knowledge of the clinical and serological profiles of the patients. Examination of testicular tissue sections revealed three different patterns: [1] normal spermatogenesis ( $n = 10$ , active spermatogenesis in all the tubules), [2] spermatogenic arrest ( $n = 12$ , the germ cells in the seminiferous tubules mature only to a particular point in the sequence of spermatogenesis), and [3] Sertoli cell-only syndrome (SCO,  $n = 9$ , seminiferous tubules have reduced diameter and are lined mainly by postpubertal Sertoli cells) (Figs. 1A, 1B, and 1C).

## FIGURE 2

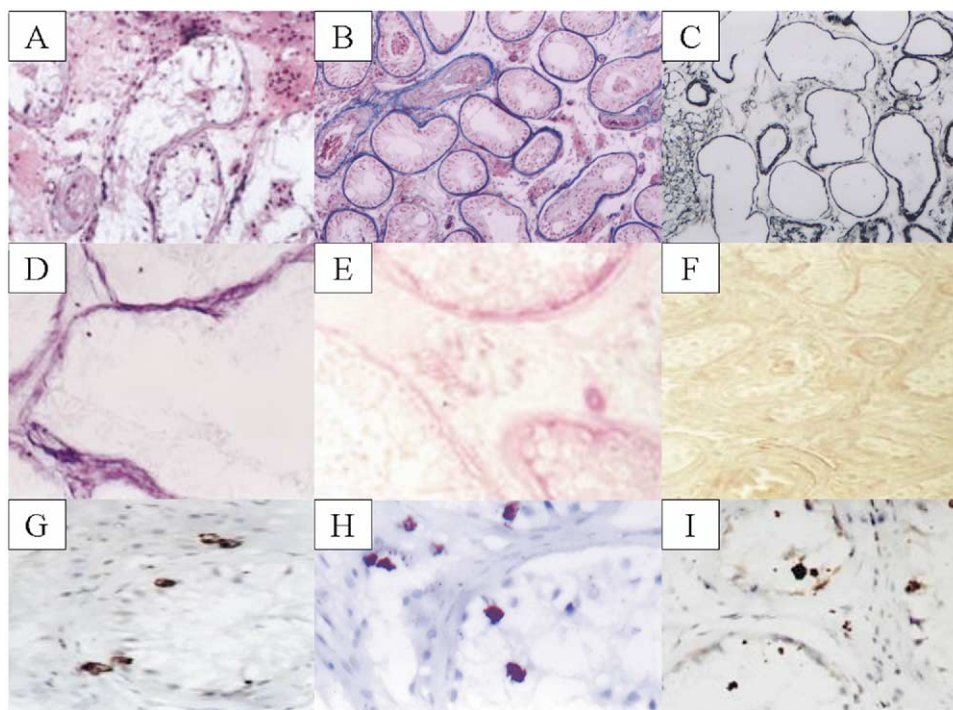
Morphological changes in spermatogenic arrest. (A) hematoxyline and eosin stain (20×); (B) Masson Trichrome stain (10×); (C) silver stain (10×); (D) Orcein stain (10×); (E) Periodic Acid Schiff's stain (20×); (F) Aldehyde Fuchsin stain (20×); (G) CD68 (macrophages, 10×); (H) CD20 (B lymphocytes, 40×); and (I) CD3 (T lymphocytes, 40×).



Hussein. Immune and mast cells in abnormal spermatogenesis. *Fertil Steril* 2005.

## FIGURE 3

Morphological changes in Sertoli cell-only syndrome. (A) Hematoxyline and eosin stain (10×); (B) Masson Trichrome stain (10×); (C) silver stain (10×); (D) Orcein stain (20×); (E) Periodic Acid Schiff's stain (20×); (F) Aldehyde Fuchsin stain (10×); (G) CD68 (macrophages, 40×); (H) CD20 (B lymphocytes, 40×); and (I) CD3 (T lymphocytes, 20×).



Hussein. Immune and mast cells in abnormal spermatogenesis. *Fertil Steril* 2005.

### Histochemical Features

In normal spermatogenesis, the seminiferous tubules were surrounded by fine collagen and reticular and elastic fibers (Figs. 1A, 1B, and 1D). In cases of spermatogenic arrest, the amount of collagen and reticular fibers increased, while that of elastic fibers decreased (Figs. 2A, 2B, and 2D). These changes were more marked in SCO cases where most of the tubules were surrounded by a very thick sheath of collagen and reticular fibers (Figs. 3A, 3B, and 3D).

Mast cells were only present in the interstitium in cases with normal spermatogenesis. Alternatively, these cells were present in the tubular walls, lumens, and interstitium in cases with abnormal spermatogenesis (spermatogenic arrest and SCO) (Figs. 1F, 2F, and 3F). The total counts of mast cells were statistically significantly higher in abnormal ( $3.81 \pm 0.15$ ) compared with normal ( $2.26 \pm 0.13$ ) spermatogenesis ( $P = .0001$ ).

### Immunohistochemical Features

A summary of our immunohistochemical findings is shown in Table 1. The CD3 and CD20 positivity appeared as brownish membranous staining, while CD68 staining ap-

peared as brownish granular cytoplasmic reactivity (lysosomes). The immune cells (T and B lymphocytes and macrophages) were present in the testes characterized by both normal and abnormal spermatogenesis (Figs. 1G–I, 2G–I, 3G–I), and were positioned in the interstitium, tubular wall, and lumens. The mean counts of cells were generally higher in cases of abnormal spermatogenesis ( $1.91 \pm 0.34$ ,  $6.01 \pm 0.93$ ,  $2.10 \pm 0.60$ , and  $3.81 \pm 0.15$  for T and B lymphocytes, CD68 macrophages, and mast cells, respectively) compared with cases of normal spermatogenesis ( $1.22 \pm 0.19$ ,  $5.41 \pm 0.58$ ,  $0.60 \pm 0.17$ , and  $0.00 \pm 0.00$  for T and B lymphocytes, CD68 macrophages, and mast cells, respectively). The differences in T lymphocyte and macrophage counts between normal and abnormal spermatogenesis were statistically insignificant ( $P = .24$  and  $P = .54$ , respectively). These differences reached the level of statistical significance only for mast cells ( $P < .0001$ ) but not for B lymphocytes ( $P = .03$ ).

Comparing normal versus abnormal spermatogenesis, both T and B lymphocytes were less frequent in the tubular walls/lumens ( $0.77 \pm 0.19$  vs.  $0.97 \pm 0.28$  and  $3.13 \pm 0.50$  vs.  $3.60 \pm 0.55$  for T and B cells, respectively) and in the interstitium ( $0.53 \pm 0.07$  vs.  $0.93 \pm 0.15$  and  $2.27 \pm 0.49$  vs.

**TABLE 1****Mean values of mast and immune cells in normal and abnormal spermatogenesis.**

| Aspect                     | T lymphocytes | B lymphocytes | Macrophages | Mast cells  |
|----------------------------|---------------|---------------|-------------|-------------|
| Complete spermatogenesis   |               |               |             |             |
| Tubular wall and lumens    | 0.77 ± 0.19   | 3.13 ± 0.50   | 0.60 ± 0.17 | 0.00 ± 0.00 |
| Interstitial               | 0.53 ± 0.07   | 2.27 ± 0.49   | 1.02 ± 0.21 | 2.26 ± 0.15 |
| Total                      | 1.22 ± 0.19   | 5.41 ± 0.58   | 1.55 ± 0.33 | 2.26 ± 0.13 |
| Spermatogenic arrest       |               |               |             |             |
| Tubular wall and lumens    | 1.06 ± 0.40   | 2.98 ± 0.66   | 1.70 ± 0.61 | 1.89 ± 0.18 |
| Interstitial               | 0.96 ± 0.20   | 2.04 ± 0.60   | 1.53 ± 0.42 | 2.38 ± 0.21 |
| Total                      | 2.03 ± 0.48   | 4.70 ± 1.00   | 2.61 ± 0.70 | 4.18 ± 0.13 |
| Sertoli cell only syndrome |               |               |             |             |
| Tubular wall and lumens    | 0.80 ± 0.30   | 5.20 ± 0.61   | 0.73 ± 0.17 | 0.84 ± 0.11 |
| Interstitial               | 0.86 ± 0.26   | 3.44 ± 0.81   | 1.53 ± 1.04 | 2.86 ± 0.16 |
| Total                      | 1.66 ± 0.46   | 9.14 ± 1.30   | 2.26 ± 1.68 | 3.35 ± 0.23 |
| Abnormal spermatogenesis   |               |               |             |             |
| Tubular wall and lumen     | 0.97 ± 0.28   | 3.60 ± 0.55   | 1.47 ± 0.47 | 1.26 ± 0.19 |
| Interstitial               | 0.93 ± 0.15   | 2.45 ± 0.50   | 1.53 ± 0.38 | 2.60 ± 0.14 |
| Total                      | 1.91 ± 0.34   | 6.01 ± 0.93   | 2.10 ± 0.60 | 3.81 ± 0.15 |

Note: All values are mean ± SD.

Hussein. Immune and mast cells in abnormal spermatogenesis. *Fertil Steril* 2005.

2.45 ± 0.50 for T and B cells in normal and abnormal spermatogenesis, respectively). CD68 macrophages were higher (not statistically significant) in the interstitium than in the tubular walls/lumens in normal (1.02 ± 0.21 vs. 0.60 ± 0.17;  $P < .05$ ) and abnormal spermatogenesis (1.53 ± 0.38 vs. 1.47 ± 0.47;  $P < .05$ ) (Table 2).

## DISCUSSION

Our knowledge about the possible relationship between abnormal spermatogenesis and both the testicular immune and mast cells is still incomplete. We hypothesized that abnormal spermatogenesis is associated with alterations in the immune and mast cells. To test our hypothesis, we carried out the current investigation. To achieve our goals, we examined the clinicopathological and immunomorphological features of testicular biopsies obtained from 31 men with azoospermia. Our study clearly demonstrates the following observations: [1] immune (B and T lymphocytes and macrophages) and mast cells were present in both normal and abnormal spermatogenesis, [2] abnormal spermatogenesis was associated with an increase in the number of these cells when compared with normal spermatogenesis, [3] there was a marked deposition of elastic and reticular fibers in abnormal spermatogenesis, and [4] the immune and mast cells from the interstitium shifted into the tubular lumens in abnormal spermatogenesis.

### Abnormal Spermatogenesis Was Associated with an Increased Number of Mast Cells

The numbers of mast cells in the testicular tissues were significantly higher in abnormal than in normal spermatogenesis.

We propose that this increased number of mast cells in abnormal spermatogenesis is associated with increased production of the tryptase enzyme, which is a potent mitogen for the fibroblasts and can enhance their chemotaxis and activation resulting in tubular fibrosis (12, 13). Therefore, the increased number of mast cells appears to be responsible for the marked deposition of collagen fibers seen in testis with abnormal spermatogenesis. We further speculate that the thickened tubular wall would impair the exchange of nutrients and oxygen between the germ cells and the fluids in the interstitium resulting in their apoptosis (14). Thus, our findings may have possible therapeutic ramifications such as the potential use of mast cell blockers to ameliorate testicular fibrosis (15), however, further population-based studies are required to examine this possibility.

### Abnormal Spermatogenesis Was Associated with an Increased Number of Testicular Macrophages

The presence of macrophages both in the interstitium and in the tubules in testis with normal and abnormal spermatogenesis is in agreement with previous studies (6, 16–19). Although the role of these macrophages in the interstitium is well known, their role in the tubules remains unclear. In our study, the number of these cells was higher in abnormal than in normal spermatogenesis (normal spermatogenesis < spermatogenic arrest < SCO). The presence of macrophages in normal spermatogenesis coupled with the normal T levels suggests that the number and quality of the interstitial macrophages in these patients were sufficient to maintain the normal development and functioning of the Leydig cells (6, 16, 20). The presence of normal LH and FSH levels in these

Comparison of P-values of mast and immune cells between complete and abnormal spermatogenesis.

| Aspect     | T lymphocytes |              |       | B lymphocytes |              |       | Macrophages |              |       | Mast cells |              |       |
|------------|---------------|--------------|-------|---------------|--------------|-------|-------------|--------------|-------|------------|--------------|-------|
|            | Tubule        | Interstitial | Total | Tubule        | Interstitial | Total | Tubule      | Interstitial | Total | Tubule     | Interstitial | Total |
| N vs. GA   | 0.70          | 0.17         | 0.24  | 0.65          | 0.51         | 0.036 | 0.50        | 0.77         | 0.55  | 0.001      | 0.11         | 0.001 |
| vs. SCO    |               |              |       |               |              |       |             |              |       |            |              |       |
| N vs. GA   | 0.44          | 0.079        | 0.10  | 0.13          | 0.74         | 0.69  | 0.53        | 0.74         | 0.73  | 0.001      | 0.72         | 0.001 |
| N vs. SCO  | 0.95          | 0.11         | 0.32  | 0.026         | 0.22         | 0.013 | 0.56        | 0.46         | 0.42  | 0.0048     | 0.028        |       |
| SCO vs. GA | 0.64          | 0.94         | 0.74  | 0.11          | 0.39         | 0.042 | 0.17        | 0.66         | 0.33  | 0.001      | 0.10         | 0.005 |

Note: All values are mean  $\pm$  SD.  
N = normal spermatogenesis; GA = spermatogenic maturation arrest; and SCO = Sertoli cell only syndrome.  
p < .01 was considered statistically significant (analysis of variance).

Hussein. Immune and mast cells in abnormal spermatogenesis. *Fertil Steril* 2005.

patients also suggests that the testicular macrophages were able to play their role in the regulation of the pituitary-testicular axis and hence in normal spermatogenesis (2, 21, 22).

The increase in the number of interstitial macrophages in abnormal spermatogenesis concurs with the finding of Frungieri et al. (17). This increase may be due to the enhanced mitosis of the resident macrophages or the increased recruitment of new ones (23). These findings further raise three possibilities. First, abnormal spermatogenesis may be associated with the presence of increased numbers of degenerated germ cells as well as an enhanced apoptotic activity. These two factors in turn could result in the recruitment of more new macrophages. In support of our proposition, previous studies reported that these cells are endocytically active in the removal of degenerated germ cells (24, 25). Second, the presence of increased numbers of macrophages in the face of normal T cell levels suggests that these cells are most probably nonactivated. If they were, they would affect Leydig cell function by production of proinflammatory cytokines and reactive oxygen species (20). This was not the case in our study as the hormonal profile of these patients and the morphology of Leydig cells were normal. Finally, increased numbers of macrophages may reflect immune dysfunction in patients with abnormal spermatogenesis.

#### Abnormal Spermatogenesis Was Associated with an Increased Number of B and T Lymphocytes

Lymphocytes (both B and T cells) were seen both in the interstitium and tubules in normal and abnormal spermatogenesis (4, 26–30). Under normal conditions, T lymphocytes act as a protective barrier preventing any immune response against spermatogenic cells. Our finding of an increase in T lymphocytes in abnormal compared with normal spermatogenesis may suggest the activation of the immune system. This may result from abnormal spermatogenesis being associated with leakage of autoantigens from the epididymis, that is, the breakdown of autoantigen segregation (2). Also, it may result from the breakdown of tolerance (clonal anergy) because of the expression of the costimulatory molecules, the CD80 and CD86 antigens.

In agreement with another study (31), there was a marked increase in the number of B lymphocytes. These changes are morphologically similar to those described in experimental autoimmune orchitis. Therefore, it is conceivable that abnormal spermatogenesis in our series was associated with disturbed permeability of the fine blood-testis barrier. In turn, the lymphocytes sensitized to germ cell antigens may cross this barrier and initiate a destructive response against the germ cells with the recruitment of B lymphocytes.

In conclusion, we report increased numbers of immune and mast cells in abnormal spermatogenesis. Our study has clearly demonstrated the following observations: [1] the

presence of immune (B and T lymphocytes and macrophages) and mast cells in both normal and abnormal spermatogenesis, [2] the association of abnormal spermatogenesis with the marked increase in the number of these cells when compared with normal spermatogenesis, [3] the marked deposition of the elastic and reticular fibers in abnormal spermatogenesis, and [4] the shift of the immune and mast cells from the interstitium into the tubular lumens in abnormal spermatogenesis. Further investigations are recommended to evaluate the associated genotypic alterations in these cells and their possible relation to germ cell abnormalities.

## REFERENCES

- Hedger MP. Macrophages and the immune responsiveness of the testis. *J Reprod Immunol* 2002;57:19–34.
- Pollanen P, von Euler M, Soder O. Testicular immunoregulatory factors. *J Reprod Immunol* 1990;18:51–76.
- Sainio-Pollanen S, Saari T, Simell O, Pollanen P. CD28-CD80/CD86 interactions in testicular immunoregulation. *J Reprod Immunol* 1996;31:145–63.
- Yakirevich E, Yanai O, Sova Y, Sabo E, Stein A, Hiss J, et al. Cytotoxic phenotype of intra-epithelial lymphocytes in normal and cryptorchid human testicular excurrent ducts. *Hum Reprod* 2002;17:275–83.
- Benahmed M. [Role of tumor necrosis factor in the male gonad]. *Contracept Fertil Sex* 1997;25:569–71.
- 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* 1989;2:249–53.
- Aguilar R, Anton F, Bellido C, Aguilar E, Gaytan F. Testicular serotonin is related to mast cells but not to Leydig cells in the rat. *J Endocrinol* 1995;146:15–21.
- Temkin V, Kantor B, Weg V, Hartman ML, Levi-Schaffer F. Tryptase activates the mitogen-activated protein kinase/activator protein-1 pathway in human peripheral blood eosinophils, causing cytokine production and release. *J Immunol* 2002;169:2662–9.
- Abe M, Kurosawa M, Ishikawa O, Miyachi Y, Kido H. Mast cell tryptase stimulates both human dermal fibroblast proliferation and type I collagen production. *Clin Exp Allergy* 1998;28:1509–17.
- Arora VK, Singh N, Bhatia A, Rashmi, Radhakrishnan G, Jain BK, et al. Testicular fine needle aspiration cytology for the diagnosis of azoospermia and oligospermia. *Acta Cytol* 2000;44:349–56.
- Hussein MR, Sun M, Roggero E, Sudilovsky EC, Tuthill RJ, Wood GS, et al. Loss of heterozygosity, microsatellite instability, and mismatch repair protein alterations in the radial growth phase of cutaneous malignant melanomas. *Mol Carcinog* 2002;34:35–44.
- Apa DD, Cayan S, Polat A, Akbay E. Mast cells and fibrosis on testicular biopsies in male infertility. *Arch Androl* 2002;48:337–44.
- Meineke V, Frungieri MB, Jessberger B, Vogt H, Mayerhofer A. Human testicular mast cells contain tryptase: increased mast cell number and altered distribution in the testes of infertile men. *Fertil Steril* 2000;74:239–44.
- Guarch R, Pesce C, Puras A, Lazaro J. A quantitative approach to the classification of hypospermatogenesis in testicular biopsies for infertility. *Hum Pathol* 1992;23:1032–7.
- Yamamoto M, Hibi H, Miyake K. Appearance of spermatozoon after administration of mast cell blocker to a patient with azoospermia. *Hinyokika Kiyo* 1994;40:541–3.
- Bergh A. Effect of cryptorchidism on the morphology of testicular macrophages: evidence for a Leydig cell-macrophage interaction in the rat testis. *Int J Androl* 1985;8:86–96.
- Frungieri MB, Calandra RS, Lustig L, Meineke V, Kohn FM, Vogt HJ, et al. Number, distribution pattern, and identification of macrophages in the testes of infertile men. *Fertil Steril* 2002;78:298–306.
- Kern S, Robertson SA, Mau VJ, Maddocks S. Cytokine secretion by macrophages in the rat testis. *Biol Reprod* 1995;53:1407–16.
- Pollanen P, Niemi M. Immunohistochemical identification of macrophages, lymphoid cells and HLA antigens in the human testis. *Int J Androl* 1987;10:37–42.
- Hales DB. Testicular macrophage modulation of Leydig cell steroidogenesis. *J Reprod Immunol* 2002;57:3–18.
- Konrad L, Weber MA, Groos S, Albrecht M, Aumuller G. Paracrine interaction in testicular somatic cells. *Ital J Anat Embryol* 1998;103:139–52.
- Li JJ, Wang T, Wang GX. [Progresses on macrophages of male reproductive tract]. *Zhonghua Nan Ke Xue* 2002;8:449–51.
- Schlatt S, de Kretser DM, Hedger MP. Mitosis of resident macrophages in the adult rat testis. *J Reprod Fertil* 1999;116:223–8.
- Breucker H. Macrophages, a normal component in seasonally involuting testes of the swan, *Cygnus olor*. *Cell Tiss Res* 1978;193:463–71.
- Ricci G, Perticarari S, Fragonas E, Giolo E, Canova S, Pozzobon C, et al. Apoptosis in human sperm: its correlation with semen quality and the presence of leukocytes. *Hum Reprod* 2002;17:2665–72.
- Aire TA, Malmquist M. Intraepithelial lymphocytes in the excurrent ducts of the testis of the domestic fowl (*Gallus domesticus*). *Acta Anat (Basel)* 1979;103:142–9.
- Itoh M, Mukasa A, Tokunaga Y, Hiramine C, Hojo K. Suppression of efferent limb of testicular autoimmune response by a regulatory CD4+ T cell line in mice. *Clin Exp Immunol* 1992;87:455–60.
- Pollanen P, Maddocks S. Macrophages, lymphocytes and MHC II antigen in the ram and the rat testis. *J Reprod Fertil* 1988;82:437–45.
- Tompkins AB, Hutchinson P, de Kretser DM, Hedger MP. Characterization of lymphocytes in the adult rat testis by flow cytometry: effects of activin and transforming growth factor beta on lymphocyte subsets in vitro. *Biol Reprod* 1998;58:943–51.
- Wilkins BS, Williams JH, Kohler JA, Jones DB. An immunohistochemical study of testicular biopsies in childhood acute lymphoblastic leukemia: reactivity of normal testicular components and leukemic infiltrates. *Pediatr Hematol Oncol* 1992;9:297–307.
- Suominen J, Soderstrom KO. Lymphocyte infiltration in human testicular biopsies. *Int J Androl* 1982;5:461–6.