Significance of human testicular mast cells and their subtypes in male infertility


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The mast cell populations in the human testis were examined using immunohistochemical techniques in five fertile volunteers and 12 patients with obstructive azoospermia, seven patients with idiopathic azoospermia, and 30 patients with varicocele. The number of mast cells per seminiferous tubular section was significantly increased ($P < 0.05$) in the men with idiopathic azoospermia. In the normal testes, mast cells containing only tryptase were the predominant subtype. In the patient groups, the predominant subtype of mast cell was shifted to that containing both tryptase and chymase. The average number of mast cells containing both tryptase and chymase per seminiferous tubular section was significantly increased ($P < 0.05$) compared with the controls in patients with obstructive azoospermia, idiopathic azoospermia, and varicocele. The number of mast cells containing only tryptase was not increased in infertile men. The selective expansion of the mast cell population containing both tryptase and chymase may be related to spermatogenetic disorders and testicular fibrosis.

Key words: cathepsin G/heterogeneity/male infertility/mast cell/trypase

Introduction

Human mast cells (MC) are found in most major organs and tissues of the body and are the major effectors of the immediate type of hypersensitivity reaction. Recent studies have shown that human MC activate fibroblasts and promote collagen synthesis by producing and releasing fibrogenic substances. Hence, MC play a role in the pathogenesis of chronic inflammation and fibrosis (Hatamochi et al., 1985; Jordana, 1993; Qu et al., 1995; Feldmann et al., 1996; Gruber et al., 1997). MC can be divided into two subtypes based on differences in their neutral serine protease content (Irani et al., 1986). MC$_T$ contain only tryptase, whereas MC$_{TC}$ contain both tryptase and chymase in addition to other proteases, including cathepsin G (Schechter et al., 1990) and carboxypeptidase (Irani et al., 1991). This heterogeneity can express itself as differences in histochemical, biochemical, and functional characteristics. The distribution of MC$_T$ and MC$_{TC}$ depends on the tissue examined (Weidner and Austin, 1993; Irani and Schwartz, 1994) and the pathological state.

It has been shown that MC can be identified in the normal human testes, and that there is an increase in the number of MC in the testes of infertile men (Maseki et al., 1981; Nagai et al., 1992). However, the role of MC in the human testis remains unknown. Using immunohistochemical techniques, we examined the heterogeneity of MC in the human testes in an attempt to relate it to spermatogenetic disorders.

Materials and methods

Tissue specimens were obtained from the testes of 12 patients with obstructive azoospermia aged 23–39 years (mean ± SD 29.2 ± 5.2), seven patients with idiopathic azoospermia aged 30–40 years (33.7 ± 5.5), and 30 patients with varicocele aged 26–46 years (33.1 ± 4.3). Tissue specimens were collected from five healthy fertile men aged 32–46 years (38.7 ± 5.6) as controls. Among the patients with obstructive azoospermia, eight patients suffered from congenital absence of the vas deferens and four patients suffered from obstructive azoospermia after herniorrhaphy. All patients with idiopathic azoospermia showed azoospermia on first examination and did not suffer from any disease that obviously caused an obstruction of the spermatic tract. All patients in the varicocele group had clinical varicocele and showed oligozoospermia. The patients in the control group were healthy volunteers who had at least one child and showed normozoospermia. Testicular biopsies were performed after obtaining informed consent.

The specimens were divided into two pieces. One piece was fixed in 10% formalin and another in Bouin’s solution for 12 h at room temperature. The tissues were prepared in an automatic tissue processor using ascending ethanol concentrations, xylene and paraffin wax. Serial paraffin sections (4 µm thick) were mounted on glass slides for staining.

To identify the mast cell subtypes, immunostaining was performed for tryptase, and cathepsin G in place of chymase because MC$_{TC}$ contained cathepsin G in addition to chymase of which immunoreactivity was lost in formalin-fixed tissue. The formalin-fixed sections were deparaffinized in xylene through ethanol to phosphate-buffered saline (PBS; pH 7.2). To block endogenous peroxidase activity, 0.3% hydrogen peroxide in methanol was applied for 20 min. All slides were then incubated in normal goat serum (Vector Laboratories, Burlingame, CA, USA) for 40 min to block non-specific binding. Primary antibodies were added to the slides and incubated for 60 min in a moist chamber at room temperature. Biotinylated anti-rabbit immunoglobulin G (IgG) was applied and incubated for 1 h. Then avidin–biotin–peroxidase complex (ABC; Vector Laboratories) was applied and incubated for 30 min. Both incubations were carried out in a moist chamber at room temperature. The final colouring agent was diaminobenzidine tetrahydrochloride. The tissue was counterstained with Methyl Green. The primary antibodies were
Table I. Hormonal analysis in patient groups. Values are given as means ± SD

<table>
<thead>
<tr>
<th></th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstructive azoospermia</td>
<td>3.9 ± 1.8</td>
<td>2.8 ± 1.2</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>Idiopathic azoospermia</td>
<td>13.7 ± 2.7</td>
<td>8.8 ± 5.8</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>Varicocele</td>
<td>7.2 ± 4.0</td>
<td>4.2 ± 2.6</td>
<td>4.8 ± 1.4</td>
</tr>
</tbody>
</table>

The mean age in the control group was significantly higher (P < 0.05) than that in the obstructive azoospermia or varicocele group. The mean age in the varicocele group was significantly higher (P < 0.05) than that in the obstructive azoospermia group. The average FSH and LH concentrations in the idiopathic azoospermia group were higher than normal (normal FSH, 1.6–9.2 mIU/ml; normal LH, 1.8–8.4 mIU/ml; Table I). The mean value of the Johnsen's score in the control group was 8.9, which was within the 95% normal limits as described by Johnsen (Johnsen, 1970; Table II). The pathological findings in the idiopathic azoospermia group showed Sertoli cell-only or maturation arrest. The Johnsen's score in the control group was significantly higher than that in the obstructive azoospermia (P < 0.05), idiopathic azoospermia, or varicocele groups (P < 0.01). The ratio of tubules with Sertoli cell-only was significantly increased in the idiopathic azoospermia group when compared with the obstructive azoospermia (P < 0.01), varicocele (P < 0.01), or control group (P < 0.05). The ratio of tubules with sclerosis and the fibrosis index were significantly increased in the patient groups when compared with the control group (P < 0.05; Table II). The Johnsen's score was significantly negatively correlated with concentrations of FSH (r = [nbh]0.49; P = 0.0006), LH (r = [nbh]0.49; P = 0.0006), and positively correlated with testosterone (r = 0.34; P = 0.024; normal testosterone concentration, 2.7–10.7 ng/ml) in all patients (Figure 1).

Testicular tissue from a patient with varicocele was immunostained using anti-trypsin antibody (Figure 2A) and anti-cathepsin G antibody (Figure 2B). The cells positive for trypsin were MC. They were found in the interstitium and lamina propria of seminiferous tubules (Figure 2A). The cytoplasm of the MC exhibited a granular staining pattern. The cells positive for both trypsin and cathepsin G were MC. They were found in the interstitium and lamina propria of seminiferous tubules (Figure 2B). The number of MC in the testes of normal fertile men. In the group with idiopathic azoospermia, the average number of MC per seminiferous tubular section was significantly increased compared with the control group (P < 0.05). The average number of MC per seminiferous tubular section was significantly increased (P < 0.05) in the obstructive azoospermia, idiopathic azoospermia, and varicocele groups when compared with the control group. There were no significant differences in the average number of MC per seminiferous tubular section between the control and patient groups. The ratio of MC to total MC was significantly increased in the obstructive azoospermia (P < 0.01) and varicocele (P < 0.05) groups when compared with the control group. There were no significant correlations between the hormone concentrations and the numbers of MC, MC_{T}, or MC_{TC} per seminiferous tubular section (data not shown).

In the varicocele group, there was significant positive correlation between the ratio of tubules with sclerosis and the fibrosis index (r = 0.50; P = 0.011). Significant correlations were observed between the number of MC_{TC} per seminiferous tubular section and the ratio of tubules with sclerosis (r = 0.41; P = 0.022), and between the number of MC_{TC} per seminiferous tubular section and the fibrosis index (r = 0.48; P = 0.013) (Figure 3).

Discussion

It has previously been shown that MC not only play a major role in allergic immune reactions, but also in tissue remodelling.
Testicular mast cells in male infertility

Table II. Numbers of mast cells (MC), MC containing only tryptase (MC\text{T}) and MC containing both tryptase and chymase (MC\text{TC}) and pathological characteristics in control and patient groups. Values are given as means ± SD

<table>
<thead>
<tr>
<th></th>
<th>No. of MC per seminiferous tubule</th>
<th>No. of MC\text{T} per seminiferous tubule</th>
<th>No. of MC\text{TC} per seminiferous tubule</th>
<th>Ratio of MC\text{TC} to total MC (%)</th>
<th>Johnson’s score</th>
<th>Ratio of tubules with Sertoli cell-only (%)</th>
<th>Ratio of tubules with sclerosis (%)</th>
<th>Fibrosis index (%) (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ((n=5))</td>
<td>1.54 ±0.40</td>
<td>0.95 ±0.39</td>
<td>0.60 ±0.23</td>
<td>37.8 ±6.3</td>
<td>8.9 ±0.6</td>
<td>0.9 ±0.0</td>
<td>1.2 ±1.1</td>
<td>6.3 ±2.1</td>
</tr>
<tr>
<td>Obstructive azoosperma ((n=12))</td>
<td>2.00 ±0.69</td>
<td>0.88 ±0.33</td>
<td>1.12 ±0.41</td>
<td>55.6 ±9.7</td>
<td>8.2 ±0.3</td>
<td>1.1 ±2.5</td>
<td>20.7 ±14.7</td>
<td>12.1 ±2.8</td>
</tr>
<tr>
<td>Idiopathic azoosperma ((n=7))</td>
<td>2.06 ±0.39</td>
<td>0.94 ±0.39</td>
<td>1.12 ±0.40</td>
<td>55.4 ±15.5</td>
<td>7.3 ±1.3</td>
<td>92.4 ±15.4</td>
<td>44.1 ±26.7</td>
<td>44.1 ±26.7</td>
</tr>
<tr>
<td>Varicocele ((n=30))</td>
<td>1.95 ±0.54</td>
<td>0.89 ±0.26</td>
<td>1.04 ±0.43</td>
<td>53.0 ±10.6</td>
<td>7.8 ±0.5</td>
<td>0.7 ±2.2</td>
<td>18.8 ±12.0</td>
<td>11.0 ±4.4</td>
</tr>
</tbody>
</table>

*aSignificant difference (\(P < 0.05\)).

**Significant difference (\(P < 0.01\)).

Total area of fibrosis/area of seminiferous tubular sections.

Figure 1. Johnson’s score plotted against the serum concentrations of FSH, LH, and testosterone in all cases.

and fibrosis (Cairns and Walls, 1997; Gruber et al., 1997; Kofford et al., 1997), host defence against infectious diseases (Malaviya et al., 1996), angiogenesis (Blair et al., 1997), and maybe even cancer invasion (Kankkunen et al., 1997). MC contribute to these processes by producing and secreting bioactive mediators. MC heterogeneity is characterized by differences in protease content, cytokine content (Bradding et al., 1995), and by electron microscopic findings (Craig et al., 1988). MC\text{T} contain tryptase, interleukin (IL)-4, IL-5, and IL-6. MC\text{TC} contain tryptase, chymase, cathepsin G, carboxypeptidase, and IL-4. MC\text{T} have secretory granules containing discrete scrolls, whereas MC\text{TC} have granules with grating and lattice substructures.

Recent studies have shown that a large number of human MC can be generated from human cord blood mononuclear cells cultured in the presence of stem cell factor and IL-6 (Saito et al., 1996; Igarashi et al., 1996). At first, cultured MC contained only tryptase (MC\text{T}). Subsequently, MC positive for chymase (MC\text{TC}) appeared. Finally, 25% of the total were mature MC\text{TC} (Igarashi et al., 1996). Using a co-culture system with murine 3T3 fibroblasts and cord blood cells, almost all of the cultured MC were changed into MC\text{TC} (Furitsu et al., 1989; Mitsui et al., 1993). It has been suggested that fibroblast-derived factors, stem cell factor, and other mediators may be required for the development of MC.

The development of MC subtypes depends on the specific tissue environment. In addition, the ratio of the subtypes changes in disease conditions. MC\text{T} are the predominant subtype in the normal gastrointestinal mucosa, nasal mucosa, and lung alveoli, whereas MC\text{TC} are the predominant subtype in the normal gastrointestinal submucosa, nasal submucosa, and skin (Weidner and Austin, 1993; Irani and Schwartz, 1994). A selective increase in MC\text{T} has been found in the skin of patients with atopic dermatitis (Irani et al., 1989), in the nasal submucosa of patients with allergic rhinitis (Bentley et al., 1992), and in breast cancer tissue (Kankkunen et al., 1997). On the other hand, an accumulation of MC\text{TC} has been found in the skin of patients with mastocytosis, which is characterized by a lack of inflammatory infiltration (Irani et al., 1990). These observations suggest that the MC\text{T} subtype is involved in allergic and inflammatory responses, whereas the MC\text{TC} subtype is involved predominantly in fibrosis and tissue remodelling.

In the testes, MC are detected in the interstitium and the lamina propria. Using histochemical procedures to identify
the proteoglycans of MC, Nagai et al. (1992) have shown that the number of MC is increased and the ratio of MC subtypes is changed in idiopathic azoospermia and oligozoospermia. In the present study, we detected the MC subtypes using immunohistochemical techniques to examine the heterogeneity in diseased human testes. Immunostaining was performed using formalin-fixed tissue. Although Bouin’s solution is commonly used for fixation of the testicular specimens, it is not suitable for immunostaining due to the destruction of the specific antigen. To identify MC TC, we used the antibody against cathepsin G which is present in MC TC because the immunoreactivity of chymase is lost in formalin-fixed tissue. Since cathepsin G is also contained in monocytes and neutrophils, it is not specific for MC (Schechter et al., 1990). Therefore, we identified the MC TC as those cells which were positive for both tryptase and cathepsin G.

We have shown that MC T are the predominant subtype in the normal testes. In contrast, MC TC were the predominant subtype in the patients with obstructive azoospermia, idiopathic azoospermia, and varicocele. The numbers of MC TC in the patient groups were significantly increased, while the numbers of MC T did not change. In the patients with idiopathic azoospermia, the number of MC TC was significantly increased.

![Figure 2](image2.png)

**Figure 2.** Testicular tissue from a patient with varicocele. (A) Mast cells (MC) were stained for tryptase in the interstitium. (B) Immunostaining of cathepsin G. The cells stained for both tryptase and cathepsin G were MC TC (arrows). The cells stained for only cathepsin G were considered monocytes (arrowheads). (C) Immunostaining of cathepsin G. MC TC (arrow) and monocyte (arrowhead) show staining at a greater magnification than in (B).

![Figure 3](image3.png)

**Figure 3.** The ratio of tubules with sclerosis and the fibrosis index plotted against the number of mast cells containing both tryptase and chymase (MC TC) per seminiferous tubular section in the patients with varicocele.
azospermia, the number of MC was significantly increased. These findings are largely the result of a selective increase in MCTC. Thus, the change in MC subtypes seems to be the result of increased infiltration of progenitor cells and of increased development of MCTC, rather than an alteration in the subtypes of the MC already present.

As previously mentioned, MCTC appear to be related to the pathogenesis of fibrosis and tissue remodelling without inflammation. In the testes of infertile men, one of the main historical changes is fibrosis in the interstitium and lamina propria of the seminiferous tubules. In the present study, increases in the ratio of tubules with sclerosis and the fibrosis index were found in the patient groups, in which a selective expansion of the MCTC population was also found. In the varicocele group, significant positive correlations were found between the number of MCTC and the ratio of tubules with sclerosis, and between the number of MCTC and the fibrosis index. These results suggest that the proliferation of MCTC increases in proportion to testicular fibrosis.

In conclusion, increases in the number of MCTC and in the MCTC to MC ratio were found in the testes of patients with obstructive azospermia, idiopathic azospermia, and varicocele. The total number of MC was increased in the testes of patients with idiopathic azospermia. The ratio of tubules with sclerosis and the fibrosis index increased in the patient groups. Significant correlations between the number of MCTC and the ratio of tubules with sclerosis, and between the number of MCTC and the fibrosis index were found in the varicocele group. A selective expansion of the MCTC population and an increase in the number of MC are related to spermatogenetic disorders and testicular fibrosis.

References

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