

The role of mast cells in male infertility

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Increased numbers of mast cells (MCs) were described in the testes of males exhibiting infertility many years ago. Since beneficial effects of treatment with MC blockers on impaired male fertility were reported, more attention has been drawn on the role of MCs in the male reproductive tract. The main interest is focused on testicular MCs, however MCs also occur in the epididymis and seminal fluid, which may be relevant for fertility as well. The increase in testicular MCs in close contact to the seminiferous tubules indicates a relationship between MC proliferation and a dysfunction of the blood–testis barrier. Activated MCs not only coincide with fibrotic events, but also with elevated numbers of several types of immune cells in the testes of infertile men and may, therefore, be involved in the pathogenesis of testicular inflammatory processes as well. Outside the testis, MCs have really been assigned a key role in chronic prostatitis/chronic pelvic pain syndrome. The occurrence of MCs in the seminal plasma of fertile/infertile men and negative effects on sperm functions has not been clarified so far and require further investigation. Optimistic reports on the beneficial effects of the treatment with MC blockers on disturbed male fertility also warrant further confirmation.

KEYWORDS: genital tract inflammation • male infertility • mast cell • spermatogenesis • testis

Mast cells (MCs) were first described by Paul Ehrlich in 1878 (Mastzellen; well fed cells), and they are now considered to play a multi-functional role in the immune system [1]. MCs commonly occur in all connective tissues and in the mucosa of many organs including skin, airways and GI tract. In male reproductive organs, MCs were first described in the lamina propria of seminiferous tubules [2], but they are also found in the interstitial compartment of human testis, the epididymis and the excurrent ducts [3–5].

Mast cells are effector cells of the immune system. Apart from their crucial role in mediating anaphylactic reactions in allergy, they are also critically involved in the pathophysiology of fibrotic disorders [6]. Studies have shown that human MCs activate fibroblasts and promote collagen synthesis by secreting fibrogenic substances, such as tryptase and chymase [7,8]. In addition, there is growing evidence for a role of MCs in host immune defense and autoimmunity [9,10]. MCs act in concert with other immune cells via a variety of receptors and cytokines, including TNF- α , granulocyte–macrophage colony-stimulating factor (GM-CSF), TGF- β , IFN- γ and several interleukins (ILs), which are expressed and secreted after antigen uptake and presentation [11,12]. Hence, MCs contribute

to immunological reactions by affecting lymphocyte growth, recruitment and function [12]. Many studies have shown that MCs also play a crucial role in innate immunity [13,14]. These data indicate that MCs function not only as sentinels but also as modulators of innate and adaptive immune responses, ultimately influencing disease outcomes.

In this article, some basic principles of MC biology and recent advances in our understanding of possible functions of MCs in the male reproductive tract were reviewed, particularly with regard to male infertility. Furthermore, the potential benefit of treatment with MC blockers was discussed. Finally, a perspective on the future developments was provided in the treatment of male infertility, that is, disorders related to infection and/or inflammation.

Basic biology of MCs

It is well known that MCs arise from CD34⁺ hematopoietic progenitor cells and initiate their differentiation in the bone marrow [15]. The precursor cells circulating in the blood enter mucosal or connective tissue where they complete their differentiation under the influence of the c-kit ligand (stem cell factor, SCF), IL-3 and the Th2 cell-associated cytokines IL-4 and IL-9,

and probably many other factors [14,16,17]. In addition to SCF, it is thought that chemokines and their receptors play key roles in the regulation of MC migration. Although studies demonstrated that there is a broad spectrum of chemokine receptors expressed at MC progenitors and mature MCs, the chemokine receptor CXCR2, but not CCR2, CCR3 or CCR5, seems to be required for the constitutive movement of MC progenitors from the blood into the small intestine in mice, a process that also requires $\alpha 4\beta 7$ integrin interaction with mucosal addressin cellular adhesion molecule (MadCAM)-1 [18].

Mast cells have the potential to secrete diverse biologically active products upon exposure to a variety of immunological or non-immunological stimuli [1]. Generally speaking, three distinct pathways of MC activation have been demonstrated: activation by fragment c receptors (FcRs), for example, high affinity receptor for IgE (Fc ϵ RI); activation by pathogen-associated substances; or activation by endogenous inflammatory factors [12]. The most notable consequence of MC activation is degranulation, which leads to the release of a spectrum of inflammatory mediators, some of which are preformed and stored in cytoplasmic granules while others are synthesized *de novo* [19]. Preformed mediators within MCs include histamine, heparin, serotonin, tryptase, chymase and TNF- α ; while prostaglandins (PGs), leukotrienes and cytokines (e.g., IL-8) are synthesized *de novo* after MC activation [12,20]. Furthermore, activated MCs are also a rich source of chemokines that may be critical for initiating and maintaining leukocyte recruitment during the inflammatory cascade. Recent data showed that bone marrow-derived MCs can produce the chemokines CCL2, CCL17 and CCL22 in response to stimulation with SCF and/or IgE [21,22].

The distribution pattern of MCs is often scattered around blood vessels and is found in the mucous layer of the GI and respiratory tracts, the skin, the testis and in close proximity to peripheral nerves [23]. Studies in rodents demonstrated that MCs can be divided into two subtypes: connective tissue and mucosal [24]. Apart from morphological and functional differences, these subtypes are characterized by the synthesis of distinct proteases: mast cell protease type I, produced by connective tissue MCs and mast cell protease type II by mucosal MCs [25,26]. By comparison, human MCs are described as either mast cells containing tryptase and chymase (MC_{TC}), or mast cells containing tryptase only (MC_T) [22,27]. The distribution of MC_{TC} and MC_T depends on the tissue examined and the pathological state. For example, MC_{TC} are associated primarily with the skin, while MC_T dominate in mucosal tissue [7,19]. The heterogeneity of MCs represents individual mast cell population that could exhibit differences in biochemical, histochemical and functional characteristics [22,28], which probably facilitate the many physiological, immunological, inflammatory or other biological reactions where this cell participates [1].

General aspects of male infertility

Male infertility is a widespread problem and can be referred to a number of causes: apart from hypothalamic–pituitary disorders, a variety of testicular diseases like cryptorchidism, testicular

injury, varicocele or testicular tumours have to be considered. In addition, disturbances at the post-testicular level, in particular epididymal dysfunctions, can contribute to disturbed male fertility (TABLE 1). Notably, infections and inflammatory disorders of the male genital tract represent a major cause of male infertility and prevalence rates of 10–15% have been reported among men undergoing andrological examination in infertility clinics [29,30]. Our previous studies demonstrated that orchitis and epididymo-orchitis due to local or systemic infection as well as noninfectious factors were important etiological reasons for male infertility [31,32]. Furthermore, spermatogenesis often was disturbed and sperm number and quality irreversibly decrease under chronically testicular inflammatory conditions. However, unfortunately, difficulties occur in the diagnosis of chronic orchitis owing to its mostly asymptomatic course and unspecific diagnostic criteria [31].

On the other hand, pathological studies of testicular biopsies indicate there is a high prevalence with inflammatory responses from infertile men [31,33]. Histopathological alterations in the damaged testis are manifested by increased numbers of leucocytes in the interstitium, autoantibody production, different degrees of germ cell degeneration and sloughing resulting in aspermatogenesis. Furthermore, testicular T lymphocytes play an important role in the pathogenesis of male infertility due to infection and inflammatory conditions [31]. Hence, these observations elucidate the fact, that despite the immune privilege of the testis protecting germ cell antigens from autoimmune attack, the testicular environment does not preclude inflammatory reactions and recruitment of tissue-specific T lymphocytes as a crucial component of the inflammation cascade [23,33,34]. In fact, testicular inflammatory disorders leading to impairment of spermatogenesis are thought to be an important reason for male infertility [30–32,35]; however, the latest studies are scarce and are not available on epidemiological data of male infertility. Similarly, the data are in line with evidence obtained from animal models, for instance experimental autoimmune orchitis [36–38].

MCs in the male reproductive tract

Testicular MCs

Testicular MCs in mammals have been found in mice, rats, dogs, cats, deer and bulls, often associated with blood vessels or close to the tunica albuginea [39]. A wide distribution of human testicular MCs, by comparison, is revealed in the interstitial tissue and within the tunica albuginea, with numbers increasing in infancy, decreasing during childhood and increasing again at puberty [5]. Increased numbers of MCs have been described in the testes of men with fertility disturbances [27,40,41], and speculations of their potential role in this context have been provided. Both data obtained from experimental animal models as well as investigations in humans suggest that, in addition to lymphocytes and macrophages, MCs may be involved in testicular and post-testicular inflammatory disease [23,42,43].

As mentioned earlier, human MCs contain several proteases and can be separated into MC_{TC} and MC_T [27]. Both MC types have also been detected in the testis and epididymis [23,44,45]. The release of tryptase by MCs in the testis has been associated with fibrotic

remodeling in defective spermatogenesis of infertile men [4,27,46,47]. Notably, fibrotic thickening of the lamina propria of the seminiferous tubules, a more frequent finding in patients with more severe fertility disturbance compared with those without [48,49], is often associated with increased numbers of activated MCs. Similar changes in testicular MC numbers have been observed in patients with spermatogenic arrest and Sertoli cell-only syndrome with maximal MC density in the latter condition, suggesting a potential role of MCs in the pathophysiology of testicular disorders [4,40,42,47,50,51]. The increase in MCs in close contact to the seminiferous tubules, even within the lamina propria, indicates a relationship between MC proliferation, differentiation and the local microenvironment, resulting in deterioration of the complex regulation and fine-tuning of spermatogenesis [52]. In early reports, the morphological observation of 'globoid' MC in the interstitial compartment versus elongated, 'fascicular' MC in the peritubular tissue and within the lamina propria was already related to a putative functional heterogeneity of testicular MC [47,50]. This hypothesis is supported by studies demonstrating increased numbers of chondroitin sulphate-positive MC or MC_{TC}, respectively, in testicular biopsies from infertile men [27,53]. Furthermore, significant levels of tryptase were demonstrated in the seminal plasma of patients with fertility disturbances [54]. However, considering the compartments of the male genital tract and volumes of primary intratubular fluid in the testis, elevated concentrations of seminal tryptase may also be referred to secretion by MCs located further downstream in the epididymis and seminal plasma. Details of these putative sources of tryptase in the male efferent duct system have not yet been clarified.

As an agonist of the proteinase-activated receptor 2, the major MC product tryptase may exert proinflammatory effects; furthermore, it is known to be involved in fibrosis and angiogenesis [43,55,56]. There is suggestive evidence that key steps of tryptase action play a pathophysiological role within the testis of infertile patients [6].

Our preliminary studies demonstrated that the selective expansion of MC_{TC} subsets was found in testicular biopsies from infertile men revealing impaired spermatogenesis and chronic inflammation compared with specimens of damaged testes without inflammation or control samples of normal, noninflamed testicular tissue. By contrast, the number of MC_T does not significantly differ in the three groups. Hence, MC_{TC} could be involved not only in the spermatogenetic disorders and testicular fibrosis but also in chronic inflammatory reactions in human testis [DUAN Y-G, CHEN S-J, HAIDL G, ALLAM J-P, UNPUBLISHED DATA].

While human MCs secrete a plethora of inflammatory mediators, so far only tryptase and chymase have been found in human testicular MCs [27,46]. Whether MCs could secrete another prototype product, namely histamine (H), and whether H receptor-bearing target cells express in the human testis remains unknown. Studies have demonstrated that testicular MCs are potential producers of H receptors H1 and H2, which are present in all compartments of human testis, despite the status of fertility. If human testicular MCs could be potential producers, H is most likely to interact with multiple testicular target cells after MC activation. Thus, further studies are needed to investigate the

Table 1. Causes of male fertility disturbances.

Cause	Ref.
Endocrine disorders: hypogonadism	[29,30]
Genetic: Y-chromosome deletions	[29,85]
Congenital: testicular maldescent	[86]
Infections/inflammation	[29,31,32]
Immunological factors	[33,34]
Toxic: social and medical drugs, occupational and environment chemicals, ionizing radiation, heat	[87]
Testicular torsion, injury	[88]
Varicocele	[89,90]
Testicular tumors	[91]
Epididymis dysfunction	[32,48]
Obstruction of the excurrent ducts	[90]
Emission and ejaculatory disturbances (including erectile dysfunction, hypospadias and so on)	[92,93]

precise nature of H1/H2 receptor-bearing cells in human testis. Data have showed that connective tissue cells, vascular cells, peritubular cells and immune cells respond to H, besides the germ cells and Leydig cells [57]. As H plays a major role in inflammation, allergy and fibrotic processes, activated MCs not only coincide with fibrotic events but also with infiltration of nonresident immune cells in the testes of infertile men [6,58,59]. The results indicate that MC-producing H might be involved in the pathogenesis and pathophysiological responses of testicular inflammatory process [57]. Furthermore, previous data have demonstrated that human MCs upregulated cyclooxygenase 2, which is the inducible form of the key enzyme during the synthesis of PGs, and production of PGE2 in fibroblasts [43,56,60], a further link to the involvement of MCs in the inflammatory testicular processes.

Recently, Th17 cells have been identified as a distinct lineage of CD4⁺ T cells that is characterized by the production of IL-17A in the inflamed testis of azoospermic patients. Interestingly, it was shown that IL-17A was expressed not only in normal testis but especially in testicular damage seen in azoospermic patients, reflected by increased numbers of positive cells and expression intensity. The baseline expression of IL-17A in normal testis suggests that IL-17A might be involved in the maintenance of testicular immune privilege and normal spermatogenesis [61]. There is compelling evidence that cytokines play an important regulatory role in the development and normal function of the mammalian testis [59]. For example, proinflammatory cytokines including IL-1 and IL-6 have direct effects on germ cell differentiation and testicular steroidogenesis under physiological conditions [62]. Local or systemic perturbation of these factors due to inflammation or infection can negatively affect testicular function, and subsequently affect male fertility [59].

Taking into account that MCs are a source of IL-6, they may also be involved in the pathogenesis of testicular inflammatory processes by interaction with Th17 lymphocytes, CD68⁺

macrophages, and CD11c⁺ dendritic cells (DCs) that are also increased in chronically inflamed testis [6,38,57,63]. As antigen-presenting cells (APCs), the latter cells play an important role in both the maintenance of immune privilege of the normal testis and the initiation and orchestration of the adaptive immune responses involving helper and cytotoxic T and B lymphocytes as effector cells [23,38,61,63]. Increased numbers of IL-23-producing CD68⁺ macrophages and CD11c⁺ DCs as well as Th17 cell-supporting cytokines (TGF- β_1 , IL-6, IL-21 and IL-22), some of them also secreted by MCs [1,9], indicate that factors present in inflamed testes might facilitate the development and effector function of Th17 cells supporting an autoimmune outcome of testicular inflammatory reactions. Furthermore, data from experimental animal models support the concept that MCs, macrophages and DCs are involved in the regulation of steroidogenesis, Sertoli cell activity and/or germ cell survival under pathological conditions including infertility due to infection and/inflammation [6,23,64].

MCs in the prostate & seminal vesicle

Although MCs have long been identified in the prostate and seminal vesicles of rats [65] and humans [66], little attention is given to phenotypic and functional properties of MCs in accessory glands under pathologic conditions. Early studies showed that there was an increase in the number of testicular MCs in rats after neonatal treatment with estrogen, whereas significant differences in the prostate and epididymis were lacking [65]. Other data suggest that prostate MCs increase in number in prostate hyperplasia and could be important players in chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) [67–69]. MCs degranulate and promote inflammation both under stress-invoked neurological and food-related allergic stimuli [68,69]. Furthermore, MC numbers increase in the periprostatic vein plexus and express tryptase, chymase and a profibrinolytic phenotype (tissue plasminogen activator-positive, urokinase-type plasminogen activator receptor-positive, plasminogen activator inhibitor-negative), suggesting that MC-derived molecules play a role in endogenous fibrinolysis [69].

An increased nerve fiber density, the sensory neuropeptide calcitonin gene-related peptide, and a progressive MC degranulation with massive release of nerve growth factor were found to strictly correlate with pain in CP/CPPS and may be implicated in the neurogenic inflammation and central sensitization [70]. The fact that the increase in MC numbers precedes the emergence of the chronic inflammation is in line with the concept that MCs initiate and/or facilitate the recruitment of other leukocytes, such as neutrophils and macrophages, as well as T lymphocytes [71,72]. Interestingly, a recent report shows that ethanol can increase the total number and degranulation of MCs in the prostate and epididymis of rats, as well as associated with an increase of mucosal MCs; however, these effects did not promote significant changes in the testis [73].

MCs in the ejaculate

There are conflicting data regarding the occurrence of MCs in the ejaculate of fertile or infertile men and the negative effects on sperm functions. Furthermore, the role of MCs in the ejaculate has not been clarified so far, and much more detailed research

is needed. While a significant increase in MC-positive samples was reported in patients with varicocele or idiopathic asthenozoospermia [74], other studies could not confirm correlations between high numbers of seminal MCs and basic semen parameters [75]. Possibly, different methods of determination of MCs (toluidine blue-pyronin staining vs flow cytometric assessment) may contribute to these controversial results. On the other hand, *in vitro* studies revealed inhibition of sperm motility after addition of tryptase via activation of the mitogen-activated protein kinase pathway [54]. The detected concentrations of tryptase in the seminal plasma, however, were far below the concentrations shown to decrease sperm motility *in vitro*. It was shown by the same authors that tryptase occurs in significant levels not only in seminal plasma but also in follicular fluid, and that MC_T are also present in the wall of the fallopian tubes. The inhibition of sperm motility by tryptase and the detection of proteinase-activated receptors on spermatozoa led to the conclusion that tryptase could directly interact with spermatozoa during their migration through the female genital tract, and that genital tract MCs and their products may be as yet unrecognized factors in human fertility and/or infertility [76].

Treatment with MC blockers

Mast cells represent effector cells of the immune system involved in inflammatory reactions bridging both innate and adaptive immune responses, which might play a role on male reproductive organs, especially the testis. In this context, several studies for treatment of patients suffering from oligoasthenozoospermia with MC blockers were found to have an improvement of standard semen parameters. 25 years ago, positive effects on semen parameters were reported after therapy with the MC blocker ketotifen. In this study, 17 men with idiopathic oligozoospermia and 22 men with idiopathic asthenozoospermia were treated with 1 mg ketotifen twice daily. After 3 months, a moderate, but statistically significant improvement of sperm count and motility had been achieved with pregnancy rates being within the range of spontaneous conceptions [77]. A further report demonstrated that spermatozoa were observed in the ejaculate of a previously azoospermic patient after treatment with a MC blocker for 3 months [78]. On the basis of this experience, a placebo-controlled prospective randomized single-blind study was performed by the same authors, involving 50 men with normal serum gonadotrophins and sperm counts below 5 million per ml. Treatment was carried out with the MC blocker tranilast, at a dosage of 300 mg per day for 3 months. The results showed that significantly higher values of sperm concentration, motility and total motile sperm count were found in the treatment group compared with the placebo group. Furthermore, the pregnancy rate in the verum group was 28.6 versus 0% in the control group [79]. These results were confirmed by a later study [80]. Similar effects were observed after treatment of 15 men revealing idiopathic oligozoospermia with the MC blocker ebastine [81]. However, no benefit was seen in infertile men with azoospermia or oligozoospermia in whose testicular biopsies increased numbers of MC_T had been observed and who were treated later on with the MC blocker fexofenadine, 180 mg/day

for 4–9 months [82]. More recently, in an open, uncontrolled study enrolling 55 men with leukocytospermia and unexplained infertility, it was demonstrated that 4 weeks of treatment with ketotifen caused a sharp reduction of white blood cell count and a significant improvement of sperm motility; after 8 weeks of treatment a significant improvement of sperm morphology was observed, and these changes remained stable until at least 4 weeks after cessation of treatment [83]. In a recent communication, the effect of administration of ketotifen on the fertility of patients having undergone varicocele surgery was studied. A total of 52 patients received ketotifen (1 mg, twice daily) for 3 months whereas the control group did not get further treatment. In the treatment group, significant improvement of semen parameters, white blood cell counts and sperm chromatin integrity was observed; furthermore, the cumulative pregnancy rate significantly increased 9 months after surgery in the treatment group (41%) compared with the control group (21%) [84].

The encouraging results of the reports reviewed here indicate that treatment with MC blockers may be a promising approach for a subgroup of men with fertility disorders. However, better selection criteria for suitable patients remain to be defined. In particular, correlations between the number of seminal MCs, and/or concentrations of tryptase and/or chymase and the outcome of treatment with MC blockers should be investigated. As MC blockers seem mainly to be effective in patients with inflammatory signs, future studies should also include seminal markers of inflammation (number of seminal leukocytes, level of cytokines such as IL-6 or TNF- α). Furthermore, it should be clarified whether MCs detected in the ejaculate originate from ‘shedding’ of resident MCs within the excurrent ducts including the epithelium of the epididymal duct or represent an influx of nonresident MCs, that is, innocent bystanders. Identification of both origin and functional status of MCs within the male genital tract seems to be an important issue, because the beneficial effects of MC blocker therapy might primarily target MCs within the testis, thus rendering the evaluation of clinical selection criteria most difficult. Therefore, sufficiently controlled studies including more appropriately defined patient subgroups are urgently needed.

Expert commentary

Mast cells are found in a diverse range of male genital tracts, and an important physiological function of these cells is the recognition of pathogens and modulation of appropriate immune

responses. However, the increase in testicular MCs in close contact to the seminiferous tubules indicates a relationship between MC proliferation and a dysfunction of the spermatogenesis. By instantly releasing several proinflammatory mediators from intracellular stores and their location at the host–environment interface, MCs can coordinate immune reactions during infectious and inflammatory process. Therefore, data suggest that MCs and their mediators could be involved in promoting inflammation and, as such, treatment with MC blockers for male infertility is beneficial. The notion that regulatory missions of MCs are modulated directly by the character of the microenvironment provides several signaling pathways in which MCs might be crucial for the prevention or promotion of inflammatory reactions and a healthy or diseased immune state, both under physiological conditions and as therapeutic target cells.

Five-year view

Considering the different secretory products of MCs and their interaction with other cells of the immune system one can also imagine that treatment of inflammatory diseases of the testis and the male genital tract may be extended to the application of nonsteroidal antiphlogistics, corticosteroids and modern immunomodulatory compounds such as cytokine antagonists. Hence, much work remains to be done to explore how MC number and phenotype changes in human testis during infectious and inflammatory conditions determine how these MCs are attracted, activated and regulated and characterize in detail the mediators they secrete and the function of these mediators in the male genital tract. Furthermore, further evaluation and better characterization of inflammation/infections of the male genital tract and their impact on male fertility should also be included. Tools to resume the testicular spermatogenesis and fertility are still on our wish list to be discovered during the next 5–10 years.

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Key issues

- Mast cells (MCs) have the potential to secrete diverse biologically active products upon exposure to a variety of immunological or nonimmunological stimuli.
- Infections and inflammatory disorders of the male genital tract represent a major cause of male infertility.
- Human MCs containing tryptase and chymase could be involved in not only spermatogenetic disorders and testicular fibrosis but also in chronic inflammatory reactions in human testis.
- MCs initiate and/or facilitate the recruitment of other leukocytes, such as neutrophils and macrophages, as well as T lymphocytes.
- *In vitro* studies revealed inhibition of sperm motility after addition of tryptase via activation of the mitogen-activated protein kinase pathway.
- The beneficial effects of MC blocker therapy might primarily target MCs within the testis.

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