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Mast cell numbers negatively correlate with fibrosis in cryptorchid testes

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Abstract *Purpose:* Mast cells have been found to play a role in fibrotic processes in multiple organ systems and are increased in number in the testes of infertile men. We have reviewed the literature and to date have found no studies investigating the role of mast cells in fibrosis of undescended testis. We examined the expression of mast cells in human cryptorchid testes and compared mast cell expression with testicular fibrosis in these testes.
Methods: Testicular biopsies from cryptorchid testis were collected over 2 years. Biopsies from 78 patients were retrospectively sectioned, stained, and reviewed for the amount of fibrosis (graded 0—3) as well as mast cell number (MCN). MCNs were quantified by tryptase staining, and the average MCN per high-powered field (HPF) was determined. Statistical analysis was performed using a one-way ANOVA with a Kruskal—Wallis test and post hoc analysis with the Dunn test when significant.
Results: Larger MCNs were significantly associated with lower fibrotic indices at the time of orchidopexy. The average MCNs were 2.06, 0.86, 0.37, and 0.58 for fibrotic indices of 0, 1, 2, and 3, respectively. MCNs were significantly higher in biopsies with a fibrotic index of 0 than all other groups ($p < 0.05$).
Conclusion: Mast cell expression correlates inversely with testicular fibrosis in cryptorchid testes. Further studies correlating mast cell expression with testicular function in boys with cryptorchidism are warranted.

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Introduction

An undescended testis occurs in approximately 3.7% of boys at birth and 1.1% will still have undescended testes at 1 year of age [1]. If the testes fail to descend on their own by 9 months of age then surgery is recommended [2]. Fibrosis of the undescended testis increases significantly after 1 year [3,4]. As fibrosis replaces the functional parenchyma of the affected testis there is simultaneously an age-related decrease in germ cell number. There is no evidence whether this is a cause—effect relationship or merely an association. It is clear that fibrosis is a time-dependent consequence of untreated cryptorchidism (and possibly treated cryptorchidism), but the pathophysiology of this process is still unknown.

Mast cells activate fibroblasts and promote collagen synthesis by producing and releasing fibrogenic substances. Mast cells have been studied extensively in fibrotic processes in the skin. They are activated by both immunoglobulin (Ig)E-mediated allergic responses and by IgE-independent activators and proinflammatory cytokines [5]. In cell culture, mast cell sonicates increase fibroblast proliferation and collagen significantly in a dose-dependent fashion. Tryptase, one of the principal molecules released by mast cells, also significantly enhances fibroblast proliferation and collagen synthesis [5]. Another mechanism by which mast cells may regulate fibrosis is by activation of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs, which are active in pathways regulating growth and differentiation [6].

Mast cells are found in normal human testes and are increased in number in the testes of infertile men. Increased mast cell numbers (MCNs) were found to correlate with sclerosis and fibrosis of seminiferous tubule in adults with varicoceles [7]. Significant rises in interstitial and peritubular mast cells have been found in adults with defective spermatogenesis [8]. In these individuals, mean total and interstitial mast cells were significantly higher in cases with sclerosing histology. These data suggest that there is a correlation between MCN and fibrosis in infertile adults but the role of mast cells in cryptorchidism is unknown.

We reviewed the literature and to date have found no studies investigating the role of mast cells in fibrosis of undescended testis and decided to study this. We hypothesized that MCN would correlate with fibrosis in cryptorchid testes at the time of orchidopexy.

Materials and methods

Our institutional review board approved this study. We retrospectively reviewed the histological and clinical findings in 126 consecutive orchidopexies performed by one pediatric urologist (B.A.K.) at Albany Medical Center between 2003 and 2005. After excluding cases with torsion and those with insufficient tissue for multiple sections, there were 78 patients whose tissue could be analyzed. The medical records of each patient were reviewed and the age at the time of orchidopexy along with the location of the testis at the time of surgery as intra-abdominal or inguinal (intracanulicular or prescrotal).

The biopsy specimens were fixed in Bouin's solution, embedded in paraffin wax, and sectioned at 4 μm and previously reviewed by two independent pathologists to determine the fibrosis grade for biopsy specimens. All histological determinations were made while blinded to clinical findings. The characterization of the fibrosis and germ cell number has been validated and previously described [3]. Briefly, they were stained with hematoxylin and eosin (H&E) and Masson's trichrome. The fibrosis was categorized by the percentage of fibrosis present: 0, <5%; 1, 6—25%; 2, 26—50%; and 3, >50%. H&E slides were used to determine the germ cell number. Fifty consecutive tubules, or all of the tubules present in the entire section if there were less than 50, were assessed.

These same specimens were stained with a tryptase stain and the average MCN per high-powered field (HPF) (40× within the testicular parenchyma was determined). Three separate physicians who were blinded to the results of the fibrosis grading performed the mast cell counts (2 pathologist and 1 urologist). Ten HPFs were examined per specimen and the average MCN was calculated. Individual physician counts were then averaged together to determine the MCN for each specimen.

Because the mast cell and counts did not exhibit a normal distribution, all statistical analysis was performed using a one-way analysis of variance with the Kruskal—Wallis test and post hoc analysis with the Dunn test when significant. We also assessed the interobserver variability between physicians performing the mast cell counts with a repeated measures analysis and Wilks' lambda test. To control for the effect of age on MCN we divided the subjects into four age groups: <1, 1—3, 4—9, and 10—15 years of age and compared the mean MCN with the fibrosis grade.

Results

We evaluated 78 testes. The mean age at the time of orchidopexy was 4 years with a range of 6 months—13.5 years. Thirty-two percent of the boys were less than 1 year old and 68% were older (Table 1). The majority of the testes were found to be in the inguinal canal or prescrotal (73%) at the time of surgery and there was no correlation between location and MCN. MCN was found to be significantly higher in boys with a fibrotic index of 0 (minimal to none) than in those with fibrosis grades 1—3 ($p < 0.05$). The average mast cells numbers were 2.06, 0.86, 0.37, and 0.58 for fibrotic indices of 0, 1, 2, and 3, respectively (Fig. 1). The average

Table 1 Subjects undergoing orchidopexy for undescended testis.

Average age in yrs (range)	4 (0.5—13.5)
Age less than 1 yr (%)	25 (32)
Age 1 yr or greater (%)	53 (68)
Location of testis:	Number (%)
Intraabdominal	11 (14)
Inguinal (canal or prescrotal)	57 (73)
Ectopic	2 (3)
Unknown	8 (10)