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Mast Cells in Pterygium: Number and Phenotype

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Purpose: To investigate the pathogenesis of pterygium.

Methods: The number and phenotype of mast cells were examined in excised tissue from 35 pterygia patients and compared with those in normal conjunctival specimens obtained during cataract or other intraocular surgery.

Results: Toluidine blue staining showed that the mean number of mast cells in the pterygia specimens was twice as high as that in the normal conjunctival tissues. Immunohistochemistry with a primary antibody to tryptase, specific for mast cells, also revealed a twofold increase in the mast cell number in the pterygia specimens compared with the normal conjunctival tissues. In the pterygia, more than 94% of the tryptase-positive mast cells were found to express chymase and c-kit. Almost all mast cells in the pterygia were tryptase-positive, chymase-positive mast cells (MC_{TC}s). There was no phenotypic difference between the mast cells in the pterygia and those in the normal conjunctival tissues.

Conclusions: The MC_{TC}s appear not to be immune system–related and to have functions in angiogenesis and tissue remodeling. The increase in the number of mast cells caused by nonallergic stimulation may contribute to the pathogenesis of pterygium. **Jpn J Ophthalmol 1999;43:75–79**

Key Words: Chymase, c-kit, mast cells, pterygium, tryptase.

Introduction

Mast cells (MCs) are key cells in the allergic inflammatory response. They are known to have important roles not only in allergic-type reactions, but also in chronic inflammatory diseases with etiopathologically nonallergic reactions under various conditions commonly accompanied by fibrosis.[1] In pterygium, several investigators have noted an increase in MC, lymphocyte, and plasma cell numbers.[2–4] Numerous fibroblast infiltrations as well as the accumulation of degenerative collagen fibers and abnormal elastic fibers were also observed.[2,5] These pathological changes in pterygium indicate a chronic inflammatory condition with fibrosis. MCs are thus presumed to have an important role in the pathogenesis of pterygium.

The human MC population is composed of groups of cells that are heterogeneous with respect to structure and function.[6,7] On the basis of their content of neutral proteases, human MCs have been divided into two phenotypes. One is the tryptase-positive, chymase-negative MC (MC_T) containing tryptase but not chymase: this is the predominant type observed in alveoli of the lung and in the small intestinal mucosa. The other is the tryptase-positive, chymase-positive MC (MC_{TC}) (containing tryptase as well as chymase), which is the predominant type observed in the skin and in the small intestinal submucosa.

These two phenotypes show substantial differences in mediator content, sensitivity to the agents that activate and release mediators, and responses to pharmacologic agents. Moreover, the phenotypic characteristics of an MC population can be changed by alterations of pathological conditions.[6–8] The dis-

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tribution of MC subsets may, therefore, have important pathogenetic and therapeutic implications.[9] In the present study, the number and phenotype of MCs in pterygia specimens were examined and compared with those in normal conjunctival specimens.

Materials and Methods

Subjects

Tissue specimens from 35 primary pterygia patients and 16 normal conjunctival tissues were examined and compared. The normal conjunctival tissues were obtained from the nasal bulbar region close to the limbus during cataract or other intraocular surgery. Written informed consent was obtained from all participating patients for the use of the tissues. All pterygia were sectioned along the longitudinal axis to include from the cap (leading edge) to the basal (body) region.

Toluidine Blue Staining

Thirty primary pterygia specimens (mean age ± SD of patients, 53.4 ± 7.5 years) and 11 normal bulbar conjunctival specimens (mean age ± SD of patients, 51.5 ± 15.3 years) were prepared for morphological study and MC count. The tissue specimens were fixed in a 2.5% formalin and 1% glutaraldehyde mixture for 24 hours at room temperature, embedded in glycol methacrylate (JB-4 kit®; Polysciences, Warrington, PA, USA) and cut into 1 μm-thick sections. These sections were then stained for 10 minutes with 1% toluidine blue (pH 4.1) for the morphological study, and with 1% toluidine blue diluted in 0.5 N HCl[10] for counting the MCs. Metachromatic cells were counted as MCs.

Immunohistochemical Staining

Five primary pterygia specimens (mean age ± SD of patients, 48.8 ± 5.2 years) and five specimens of normal bulbar conjunctiva (donor ages ± SD, 61.8 ± 11.5 years) were prepared for immunohistochemical study. The specimens were fixed in Zamboni's fixative for 16 hours at 4°C, embedded in OCT Compound® (Miles, Elkhart, IN, USA), and then snap-frozen. Cryostat sections (5-μm-thick) were used for all immunohistochemical stainings. An immunohistochemical study was carried out using a labeled streptavidin-biotin technique (LSAB kit®; DAKO, Tokyo) at room temperature. The primary antibodies used in this study were monoclonal mouse anti-human mast cell tryptase (0.5 μg/mL; Chemicon, Temecula, CA, USA), monoclonal mouse anti-human mast cell chymase (0.5 μg/mL; Chemicon), and monoclonal anti-human c-kit (0.5 μg/mL; Nichirei, Tokyo); the incubations with each were performed for 2 hours. Mouse IgGl was used as a negative control. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 minutes. The chromogen used was 3-amino-9-ethylcarbazole (AEC), and counterstaining was done with hematoxylin. The tryptase-positive cell count was compared with the chymase-positive or c-kit–positive cell count in the adjacent sections. For each specimen, three sequential sections of three different regions were examined.

Counting of MCs

Each section was photographed at ×200 magnification, and the entire tissue area was examined. The MCs were identified under light microscopy at ×400 magnification, and were marked on each photograph. The number of MCs was then counted. The total area of the substantia propria (subepithelial connective tissue) except for the corneal stroma and the coagulation area was measured with a computer (Spicca II®; Nippon Avionics, Tokyo). The MC counts were calculated as cells/mm^2.

Results

Toluidine Blue Staining

All metachromatic MCs in the pterygia and the normal conjunctival tissues were found in the substantia propria. In the pterygia, only a few MCs were found at the cap area, where fibroblast infiltration was seen between basal cells of the epithelium and the Bowman's layer or around the dissolved edge of the Bowman's layer (Figure 1). Many MCs were observed around the elastotic degeneration area, and were aggregated with the lymphocytes and the plasma cells around the small blood vessels in the substantia propria at the body of the pterygia (Figure 2). The mean number of MCs in the pterygia was significantly higher than that in the normal conjunctival tissues (Table 1).

Immunohistochemical Staining

Positive staining was not observed in any of the negative controls. In the pterygia and the normal conjunctival tissues, all tryptase-positive cells and all chymase-positive cells were observed in the substantia propria. The c-kit–positive cells were detected in some of the basal cells of the epithelium as well as in the substantia propria. The mean number of tryptase-positive cells in the pterygia was significantly higher

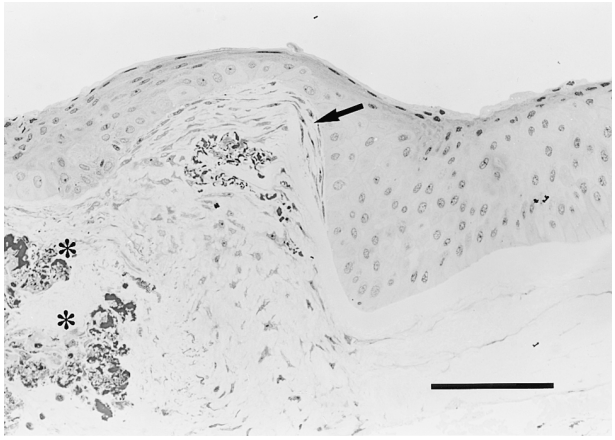

Figure 1. Cap region of a pterygium. Fibroblasts invaded between corneal epithelial cells and Bowman's layer (arrow). Here, Bowman's layer is dissolved and only a few mast cells are observed. Accumulations of elastoid materials (asterisks) are seen in substantia propria. (Toluidine blue [pH 4.1] staining.) Bar = 100 μm.

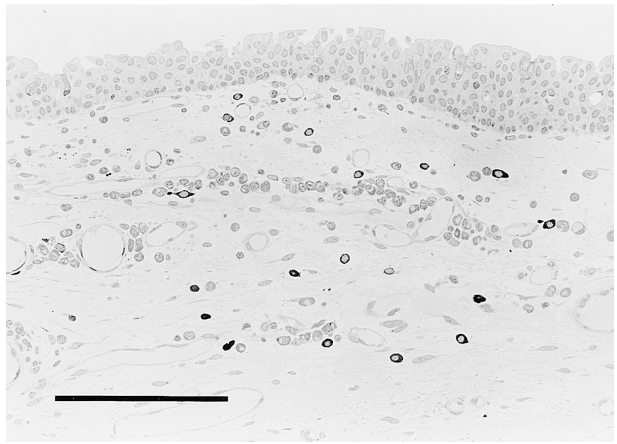

Figure 2. Infiltration of many mast cells shown by dark staining with toluidine blue diluted in 0.5 N HCl, as well as plasma cells and lymphocytes around capillaries in substantia propria of a pterygium. Bar = 200 μm.

than that in the normal conjunctival tissues (Table 1). The tryptase-positive cells were confirmed to express chymase and c-kit by the observation of adjacent sections (Figure 3). Neither the mean percentage of chymase-positive cells among the tryptase-positive cells nor that of c-kit–positive cells among the tryptase-positive cells showed any significant differences between the pterygia and the normal conjunctival tissues (Table 1).

Discussion

The pathogenesis of pterygium is still unclear. Histopathologically, the increased infiltration of lymphocytes, predominantly that of T-cells, plasma cells, and MCs are observed in pterygium.[2–4] In addition, depositions of IgE and IgG in pterygium have been reported.[11] Therefore, it has been suggested that an immunologic mechanism involving hypersensitivity contributes to the pathogenesis of pterygium.[2–4,11] The number of MC in pterygium has been reported to be about twice as high as that in normal conjunctiva in previous studies[4,12–14] in which the MCs were detected by metachromatic dye staining or by morphological characteristics. In the present study, the metachromatic cell number in the pterygia was similarly twice as high as that in the normal conjunctival tissues. We have noted here that basophils, like MCs, are also stained metachromatically, and that the degree of metachromatic staining of MCs is dependent on the fixative employed.[7] We made the above conclusion after confirming the number of MCs by counting tryptase-positive cells after immunohistochemical staining specific for MCs[15] to exclude the possible contribution of the basophils, as well as counting the MCs that failed to be stained, and adding both totals to the total metachromatic cell number. The number of MCs is known to increase in allergic and parasitic diseases. It has been reported[9,16] that the number of MCs increased in vernal conjunctivitis and allergic conjunctivitis, as compared with that in normal conjunctiva and these allergic diseases, the phenotypes of the increased MCs, were predominantly MC_Ts, whereas MC_{TC}s were the predominant type in normal conjuctiva. These two phenotypes of human MCs show different responses to pharmacologic agents. For example, MC_{TC}s are shown to be sensitive to stimulation by substance P and other basic secretogogues, but insensitive to inhibition by disodium cromoglycate (DSCG); whereas MC_Ts are insensitive to substance P, but sensitive to the inhibition of histamine release by DSCG.[6,7] This may be the reason why DSCG is efficacious in these diseases.[16] In the present study, although the number of MCs was confirmed to be increased in the pterygia, no significant difference was found in the phenotype of MCs between the pterygia and the normal conjunctival tissues, and more than 94% of the MCs were MC_{TC}s. An increase in MCs has been observed not only in allergy, but also in nonallergic chronic inflammation, angiogenesis, fibrosis, and tissue remodeling. The differences in the functional roles of the two phenotypes are not completely understood. Concerning phenotypes, however, it is recognized that MC_Ts are "immune system-

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Table 1. Cells in Pterygium and Normal Bulbar Conjunctiva

	Pterygium	Normal Bulbar Conjunctiva	*P*
Metachromatic cells (Toluidine blue staining)	34.5 ± 18.0/mm^2 (n = 30)	15.5 ± 9.0/mm^2 (n = 11)	$P < .01$[a]
Tryptase-positive cells	50.7 ± 6.9/mm^2 (n = 5)	24.1 ± 8.9/mm^2 (n = 5)	$P < .01$[b]
Chymase-positive cells among tryptase-positive cells	94.2 ± 5.3% (n = 5)	96.7 ± 4.6% (n = 5)	NS
C-kit–positive cells among tryptase-positive cells	97.8 ± 5.8% (n = 5)	103.6 ± 8.4% (n = 5)	NS

NS: not significant
Data are mean ± SD.
[a]Mann-Whitney Rank Sum Test.
[b]*t*-test.

related" MCs with a primary role in host defense, whereas MC_{TC}s that increase in fibrotic diseases are "non-immune system–related" MCs with functions in angiogenesis and tissue remodeling rather than immunologic protection.[17] This concept suggests that the MCs in pterygium increase in number as a response to nonallergic stimulation.

Stem cell factor (SCF) is recently well known to be a factor that regulates MC growth and function. It was demonstrated that SCF can regulate the migration and survival of MC precursors, promote the proliferation of both immature and mature MCs, enhance MC maturation, and directly induce the secretion of MC mediators.[18] Stem cell factor was also suggested to be one of the most important factors influencing the MC number, phenotype, and function in both health and disease conditions. An overexpression of SCF may cause the augmentation of MCs in the pterygium. The MC and MC precursor express the SCF receptor, encoded by the c-kit protooncogene.[19] The c-kit expression of MCs was demonstrated to be down-regulated when an overexpression of SCF is observed.[20,21] On the other hand, it has been reported that all MCs in many normal organs and in diseases with an augmentation of MCs, eg, allergic conjunctivitis, urticaria pigmentosa, and mastcytosis, expressed c-kit.[16,19,22] The present results demonstrated that almost all MCs in the pterygia, as well as in the normal conjunctival tissues, expressed c-kit. It is unclear whether the degree of the expression of SCF contributes to the expression of c-kit on MCs, and what conditions influence the expression of c-kit on MCs.

It is known that SCF affects melanocyte proliferation in the skin and that normal melanocytes express c-kit.[18] In the present study, c-kit expression was also observed in some basal cells of the epithelium in the pterygia and the normal conjunctival tissues. This result may have been caused by the epithelial basal cells with cytoplasmic pigmentation.

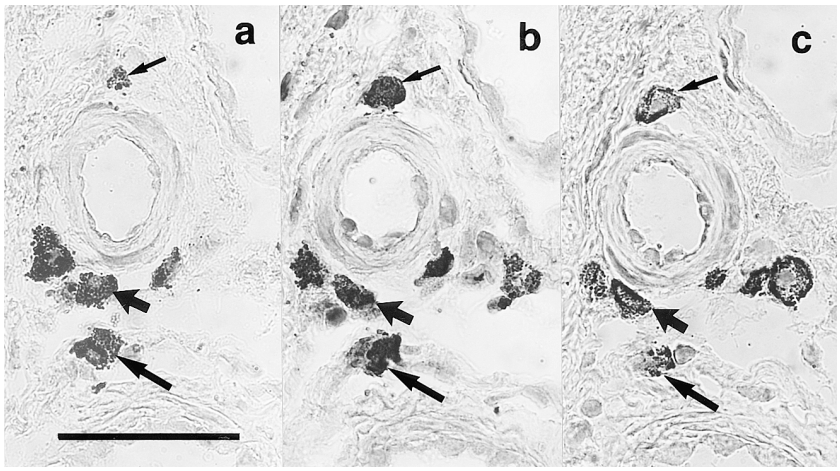

Figure 3. Immunohistochemical staining with monoclonal antibodies to (**a**) chymase, (**b**) tryptase, and (**c**) c-kit performed on adjacent sequential sections in a pterygium. Same mast cells (arrows) expressed chymase, tryptase, and c-kit. Bar = 50 μm.

In conclusion, the present study confirmed the twofold increase in the number of MCs in pterygia compared with normal conjunctival tissues by both toluidine blue staining and immunohistochemical staining with a primary antibody to tryptase. The phenotype of the increased MCs in the pterygia was MC_{TC}, and there was no phenotypical difference between the MCs in the pterygia and those in the normal conjunctival tissues. These results suggest that an increase in MCs caused by a nonallergic mechanism contributes to the pathogenesis of pterygium, since the MCs produce and secrete a large amount of biologically active mediators, and MC_{TC}s are presumed to be MCs functioning mainly in angiogenesis, tissue remodeling, and fibrosis.

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