

Ocular Mast Cells and Mediators

Anne-Marie A. Irani, MD*

Division of Pediatric Allergy, Immunology, and Rheumatology, Virginia Commonwealth University Health System, 1112 East Clay Street, Mc Guire Hall Annex Room 4-421, Richmond, VA 23229, USA

Mast cells have long been recognized for their role in immediate hypersensitivity reactions, by virtue of the presence of high affinity receptors for IgE (FcεRI) on their surface. More recently, mast cells have been postulated to be involved in a variety of chronic inflammatory disorders as numerous mediators released by activated mast cells are characterized. This article summarizes current information on mast cell mediators, heterogeneity, and differentiation, and it reviews studies of mast cells in the normal eye and various ocular disorders.

Mast cell mediators

The humoral and cellular inflammatory responses occurring during immediate hypersensitivity reactions are initiated by numerous mediators of mast cells and possibly by basophils. Some mediators are stored preformed inside the secretory granules of mast cells and are released rapidly upon mast cell activation by a process of regulated secretion or degranulation. Other mediators are synthesized *de novo* upon mast cell activation and are released without a prolonged intracellular storage phase after variable time intervals. In humans, the principal preformed mediators of mast cells include histamine, proteoglycans, neutral proteases, and possibly, basic fibroblast growth factor. Newly generated mediators include the arachidonic acid metabolites prostaglandin (PG) D₂, and leukotriene (LT) C₄, and TH2-like cytokines such as interleukins (IL) 4, 5, 6, and 13, and tumor necrosis factor-α (TNF-α).

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* Virginia Commonwealth University, Box 980225, Richmond, VA 23298-0225, USA.

E-mail address: airani@vcu.edu

Preformed granule-associated mediators

Biogenic amines

Histamine is stored in secretory granules of mast cells, is bound to carboxyl and sulfate groups on proteins and proteoglycans, and is the sole biogenic amine in human mast cells and basophils. Human mast cells contain 1 to 5 pg of histamine per cell [1], which is equivalent to a concentration of 0.1 M inside the secretory granules. In contrast, plasma histamine concentration averages 2 nM. Intermediate concentrations in various biologic fluids, such as nasal lavage fluid, reflect local rates of production and diffusion or metabolism. After mast cell degranulation, released histamine is metabolized within minutes. Elevated levels of histamine in plasma or urine are found following anaphylactic reactions and indicate mast cell or basophil involvement. Histamine exerts potent biologic effects through its interaction with cell-specific receptors designated H1, H2, and H3 receptors [2–4]. Stimulation of H1 receptors results in vasodilation and increased capillary permeability, contraction of bronchial and gastrointestinal smooth muscle, and increased mucous secretion at mucosal sites. H2 receptor agonists stimulate gastric acid secretion and exert numerous effects on cells of the immune system, including augmentation of suppression by T lymphocytes [2], inhibition of mediator secretion by cytotoxic lymphocytes and granulocytes, and suppression of eosinophil chemotaxis [5]. Additional effects include activation of endothelial cells to release prostacyclin, a potent inhibitor of platelet aggregation. Stimulation of H3 receptors affects neurotransmitter release and histamine production in the central and peripheral nervous system [6]. H3 receptors are postulated to mediate interactions between mast cells and peripheral nerves, and they may be involved in histamine-mediated neurogenic hyperexcitability.

Proteoglycans

The presence of highly sulfated proteoglycans in secretory granules of mast cells confers the metachromatic staining properties of these cells when stained with basic dyes, such as toluidine blue or alcian blue. In human mast cells, heparin and chondroitin sulfate E are the major intracellular proteoglycans [7,8]. Heparin is found in all mature mast cells [9], but not in other cell types. The biologic functions of endogenous mast cell proteoglycans await further clarification. These proteoglycans bind to histamine, neutral proteases, and acid hydrolases at the acidic pH found inside the mast cell secretory granules, and may play an important role in intracellular packaging. Heparin facilitates processing of chymase and tryptase forms to their active forms. The stabilizing effect of heparin and (to a lesser degree) chondroitin sulfate E on tryptase activity may be critical for mast cell physiology [10,11]. Heparin and (to a lesser extent) chondroitin sulfate E express anticoagulant, anticomplement, and antikallikrein activities.

Heparin facilitates fibroblast growth factor activity in cutaneous mast cells [12,13] and modulates the cell adhesion properties of matrix proteins, such as vitronectin, fibronectin, and laminin.

Neutral proteases

Neutral proteases cleave peptide bonds near neutral pH and are the dominant protein components of secretory granules in rodent and human mast cells [14]. These enzymes also serve as selective markers that distinguish mast cells from other cell types and distinguish different types of mast cells from one another. Trypsin is a serine class protease with tryptic-like activity [15,16]. It is stored in secretory granules bound to heparin, which is essential for trypsin to retain its enzymatic activity [10], and it is not inhibited by biologic inhibitors of serine proteases present in plasma and urine. Trypsin is present in all mast cells at concentrations that vary from 10 pg per mast cell derived from the lung to 35 pg per mast cell derived from the skin [1]. Negligible amounts have been detected in human basophils (0.04 pg/cell) [17], but not in any other cell type. Trypsin is released together with histamine during degranulation [18] and serves as a specific marker of human mast cells. At least two homologous trypsin genes, termed α and β , have been identified on human chromosome 16, and the corresponding recombinant proteins have been synthesized [19–21]. A mutation in the leader sequence of α -trypsin prevents it from processing to a mature form and packaging in secretory granules. Thus, α -protrypsin is thought to be constitutively secreted, is enzymatically inactive, and is the principal form of trypsin detected in the peripheral circulation in normal controls. Mature β -trypsin is the principal form of trypsin stored in secretory granules, is enzymatically active, is usually undetectable in normal controls, and is released during mast cell degranulation. Therefore, elevated serum levels of α/β -protrypsin reflect mast cell hyperplasia such as what occurs in systemic mastocytosis, whereas elevated serum levels of mature β -trypsin reflect mast cell activation such as what occurs in systemic anaphylaxis [22]. Because β -trypsin accounts for all of the trypsin enzymatic activity, it is the principal form measured by enzymatic assays, whereas the currently commercially available trypsin immunoreactive assay (Pharmacia, Uppsala, Sweden) measures a combination of α and β trypsinases. Potential biologic activities of trypsin include inactivation of fibrinogen, with subsequent anticoagulant effect, which explains the lack of fibrin deposition in reactions involving urticaria and angioedema, and consequently, their rapid resolution [23]. Trypsin augments histamine constriction effects on airway smooth muscles [24] and degrades vasoactive intestinal peptide (an intrinsic neuropeptide with bronchodilating properties), effects that may result in increased bronchospasm. In synovial cells derived from subjects who have rheumatoid arthritis, trypsin activates latent collagenase [25]. Trypsin stimulates growth of smooth muscle cells and fibroblasts in vitro [26,27].

The recent development of tryptase inhibitors as potential therapeutics should help better identify the physiologic role of this enzyme in vivo.

Chymase is a serine protease with chymotryptic-like activity that was purified from human skin [28], and the corresponding gene was cloned and localized to human chromosome 14 [29]. Like tryptase, chymase is bound to heparin but remains stable after dissociation from heparin [30]. Chymase activity is inhibited by the classical biologic inhibitors of serine proteases, such as α_1 -antichymotrypsin, α_1 -proteinase inhibitor, and α_2 -macroglobulin [31]. Dispersed skin mast cells contain 4.5 pg of chymase per cell, whereas neither chymotrypsin enzymatic activity nor chymase mRNA are detected in lung mast cells, thus localizing chymase to a subpopulation of human mast cells [17,32]. Potential biologic activity of chymase includes potent activation of angiotensin I [33] and procollagenase [34] and inactivation of bradykinin [35]. Chymase digests the lamina lucida of the basement membrane at the dermal-epidermal junction of human skin [36] and degrades substance P, vasoactive intestinal peptide, and probably other neuropeptides [37,38]. Dog chymase stimulates mucus production from glandular cells in vitro [39], suggesting a similar role in allergic disorders at various organ sites, such as mucous hypersecretion in asthma and allergic rhinitis and mucous hypersecretion in allergic ocular disorders.

Cathepsin G is a serine neutral protease with chymotryptic activity that colocalizes with chymase in human mast cells [28]. It also is found in neutrophils and monocytes and therefore cannot be used as a specific marker of mast cells. Human mast cell carboxypeptidase also resides with chymase and cathepsin G in a subpopulation of human mast cells [40]. It cleaves the carboxyterminal His⁹/Leu¹⁰ bond of angiotensin I and behaves like a zinc metalloexopeptidase; it is inhibited by *o*-phenanthroline [41]. Dispersed human skin mast cells contain 5 to 16 pg of carboxypeptidase per cell.

Newly generated mediators

Lipid mediators

Unstimulated human mast cells incorporate exogenous arachidonic acid into membrane and cytoplasmic lipid [42]. Upon activation of mast cells, arachidonate released by hydrolysis of these lipids is metabolized along the cyclooxygenase pathway, giving rise to PGs and thromboxanes, or (along the lipoxygenase pathway) LTs. Dispersed preparations of human mast cells obtained from lung, skin, or small intestine and subjected to activation produce PGD₂, in excess over LTC₄, and smaller amounts of LTB₄ [43]. In contrast, activated basophils produce LTC₄, but no PGD₂. The production of arachidonic acid metabolites begins within minutes of cell activation and lasts up to about 30 minutes. These mediators also are produced by many other cell types, and their detection in biologic fluids cannot be used as a specific marker of mast cell or basophil activation. Relative

production of these lipid mediators cannot be used to distinguish various types of mast cells. The biologic activity of arachidonic acid metabolites has been underscored by the favorable clinical effects of 5-lipoxygenase inhibitors in atopic and aspirin-induced asthma, where mast cell activation occurs [44]. PGD₂ production may be involved in the pathogenesis of recurrent hypotensive episodes in a subgroup of subjects who have systemic mastocytosis, as evidenced by clinical improvement following inhibition of cyclooxygenase with aspirin [45].

Cytokines

Upon activation, human mast cells produce an array of cytokines, including up-regulation of the IL-4 gene cluster on human chromosome 5 (IL-4, IL-5, IL-6, IL-9, IL-13, and granulocyte macrophage-colony-stimulating factor), and of TNF- α on human chromosome 6 [46–48]. Mast cells preferentially produce IL-13 over IL-4 as opposed to basophils, which produce more IL-4. Cytokine production occurs minutes to hours following mast cell activation. Cytokines derived from activated mast cells serve to activate endothelial cells to recruit eosinophils and other inflammatory cell types at the site of immediate hypersensitivity reactions, thus giving rise to the late phase of allergic response and sustained inflammation. These cytokines also may be involved in modulating the local distribution of the Th1 and Th2 types of T lymphocytes, thereby expanding the biologic role of mast cells far beyond type I hypersensitivity reactions only.

In conjunctival biopsy specimens obtained from normal subjects, and those with seasonal allergic conjunctivitis during and outside the pollen season, mast cells comprised greater than 90% of IL-4⁺ immunoreactive cells and the majority of IL-5⁺, IL-6⁺, and IL-13⁺ cells [49].

Mast cell heterogeneity

Phenotypic heterogeneity

Mast cell heterogeneity was recognized first in the rodent system with the early work of Enerback and colleagues [14], who demonstrated variations in the metachromatic staining properties of rat mast cells based on the type of fixative. Thus, mast cells in the gastrointestinal mucosa required fixation in Carnoy's fluid or in isotonic formaldehyde acetic acid to be visualized with metachromatic stains and were referred to as “mucosal mast cells” or “formalin-sensitive mast cells”. Mast cells in the skin and the peritoneal cavity were seen easily with metachromatic stains after fixation in formalin and the previously mentioned fixatives, and they were termed “connective-tissue mast cells” or “formalin-resistant mast cells”.

A similar differentiation was difficult to reproduce in the human system. Instead, differentiation of human mast cells based on their neutral protease

content was demonstrated first in 1986 [32]. At least two types of mast cells were defined in humans. MC_T cells were found to contain tryptase, but none of the other mast cell proteases, whereas MC_{TC} cells contained tryptase and chymase, cathepsin G, and human mast cell carboxypeptidase [50,51]. These proteases appear to reflect qualitative differences, as evidenced by a lack of chymase mRNA and the enzyme in morphologically mature MC_T cells [17]. Furthermore, cultured MC_T cells from lungs remained deficient in chymase protein and chymase mRNA [52]. MC_T cells were found to predominate in the gastrointestinal mucosa of the small intestine and in the alveolar lining and epithelium of the lung, whereas MC_{TC} cells predominated in the skin and in gastrointestinal submucosa of the small intestine [32]. The subepithelium of the nasal mucosa and the bronchial walls contained a mixture of both types of mast cells. More recently, mature MC_{TC} cells obtained from human lung and skin preparations were shown to express surface CD88 (C5aR) and could be separated from MC_T cells by cell sorting [52]. The relative abundance of MC_T and MC_{TC} cells varies with tissue inflammation or fibrosis [53,54], and the protease phenotype cannot be deduced based on location alone. Therefore, the nomenclature of mucosal mast cells and connective tissue mast cells appeared inadequate in this system. Mast cell heterogeneity based on differential expression of various neutral proteases was characterized later in the mouse system by the elegant experiments of Stevens and colleagues [55,56]. Variations in expressions of mouse mast cell proteases 1 through 7 were demonstrated initially at the message level and were demonstrated later at the protein level as well; variations depended on stages of maturation of the mast cells and tissue localization, suggesting that local environmental factors were involved in directing mast cell differentiation.

Mast cell heterogeneity based on differential expression of various cytokines was demonstrated first by Bradding and colleagues [57]. Skin mast cells were found to express IL-4, but little if any IL-5 and IL-6, whereas lung and nasal mast cells expressed predominantly IL-5 and IL-6 and lesser amounts of IL-4. At this time, it is unclear if the differential expression of cytokines corresponds to the different protease phenotypes, but it suggests that MC_{TC} mast cells, which are almost the exclusive type of mast cell found in the skin, express predominantly IL-4, whereas MC_T cells, which predominate in the lung, express predominantly IL-5 and IL-6. This concept was supported further by studies of conjunctival biopsies with sequential *in situ* hybridization and double immunohistochemistry; these studies demonstrated that MC_{TC} cells comprised 89.2%–93.3% of IL-4⁺ cells and 77.8% of IL-4⁺ cells in seasonal allergic conjunctivitis and normal subjects, respectively [49]. Similarly, IL-13 appeared to colocalize preferentially to the MC_{TC} cells, while IL-5 and IL-6 were more commonly expressed in the MC_T cells. Functional differences between these two types of mast cells could be postulated based on differential expression of cytokines.

Functional heterogeneity

Mast cell heterogeneity also can be defined on the basis of functional differences. Stimulation by way of the FcεRI receptor and calcium ionophores activates all tissue-derived mast cells. Nonimmunologic agonists, however, show selectivity for mast cells isolated from various tissue sites. Agents, such as morphine sulfate, substance P, vasoactive intestinal peptide, somatostatin, calcitonin gene-related protein, and the anaphylatoxins C5a and C3a, cause histamine release from skin mast cells but not from mast cells derived from the lung, the tonsils, the adenoids, and the colon, regardless of protease phenotype [58–62]. Heart mast cells respond to C5a but not to substance P [63,64]. Dispersed conjunctival mast cells are activated by morphine, but not substance P, to release low levels of histamine, tryptase, leukotrienes, and PGD₂. Conjunctival mast cells also respond to compound 48/80 [65]. Such tissue-specific differences in the mast cell secretory response may be caused by microenvironmental influences rather than to lineage. For example, the rat basophil leukemia cell line (which represents rat mucosal mast cell) is unresponsive to substance P at baseline, but responds well when cocultured with murine 3T3 fibroblasts without otherwise changing its phenotype [66]. Such microenvironmental influences may result from intrinsic changes in the mast cell brought about by accessory cells or by way of acquisition of new membrane components from accessory cells. A further level of functional heterogeneity occurs in MC_{TC} cells derived from lungs versus skin. Thus, although lung-derived MC_{TC} cells produce LTC₄ upon activation, skin-derived MC_{TC} cells do not [52].

Pharmacologic heterogeneity

Pharmacologic responsiveness of mast cells also varies depending on the tissue source. Sodium cromoglycate and nedocromil, when used at high concentrations, are weak inhibitors of activation of lung-derived mast cells, but they have no effect on mast cells from the skin and intestines [67–69]. Adrenoceptor agonists, cyclosporine A, and FK-506 produce inhibition of IgE-dependent histamine release in vitro from skin- and lung-derived mast cells [70–72]. Lodoxamide reduces the allergic response in rat conjunctiva in vivo following allergen challenge, and inhibits histamine release in vitro from rat conjunctival mast cells in a dose-dependent manner [73]. Dexamethasone in vitro does not inhibit activation-secretion by human lung-derived mast cells [74]. In vivo, topical nasal glucocorticosteroids result in diminished mediator release in response to nasal allergen challenge [75], perhaps caused by decreased mast cell concentration after prolonged topical administration as demonstrated in the skin and in the synovium [54,76]. Recent studies using IL-10 knockout mice have demonstrated a protective effect of IL-10 on degranulation of conjunctival mast cells in response to compound 48/80 [77].

Mast cell differentiation

Mast cells originate from hematopoietic stem cells and differentiate under the influence of stem cell factor (SCF), the ligand for Kit, a product of the *c-kit* proto-oncogene [78–80]. Unlike most other hematopoietic cells, mast cell progenitors circulate in the peripheral blood and complete their differentiation in peripheral tissues along a distinct myeloid lineage, which is different from that of basophils, monocytes, or other leukocytes [81,82]. Whereas other myelocytes stop expressing surface Kit as they complete their differentiation, mast cells express increasing amounts of Kit as they mature and require the persistent presence of SCF for their continued survival [79,80,83]. The critical effects of SCF in mast cell development and survival are evident particularly in certain strains of mice, with genetic defects in either Kit expression or SCF production, in which a profound mast cell deficiency occurs [84]. Although IL-3 serves as an important growth and differentiation factor for rodent mast cells [85], it appears to have little influence on human mast cells, which lack a surface receptor for IL-3 [86]. Although SCF appears to be an essential growth factor, other factors or accessory cells may be involved in regulation of mast cell development. For example, IL-4 down-regulates the expression of Kit in mast cells [87], including conjunctival mast cells [88], and results in inhibition of SCF-dependent development of mast cells from progenitors *in vitro*, but IL-4 has little effect on mature mast cells [89,90]. This effect may be responsible, in part, for the absence of mast cells in normal bone marrow despite the presence of large amounts of SCF. Glucocorticosteroids have a similar inhibitory effect on SCF-dependent mast cell development, whereas mature mast cells are relatively resistant to the effect of glucocorticoids. Furthermore, mast cells developing in cultures of fetal liver cells in the presence of SCF appear immature by morphologic criteria and lack the high affinity receptor for IgE, whereas mast cells developing from cord blood mononuclear cells cocultured with murine 3T3 fibroblasts (which produce SCF) appear mature and possess surface FcεRI. Thus, cell-associated SCF found on the surface of fibroblasts or other factors produced by fibroblasts may contribute to the maturational effect on mast cell development.

Conditions that influence the selective development of MC_T, or MC_{TC}, cells are not understood yet; however, commitment to a particular mast cell phenotype appears to occur by the time granule formation begins, so the compositional differences between MC_T and MC_{TC} cells can be appreciated at the electron microscopy level in morphologically immature cells [91], which suggests parallel rather than sequential development. However, studies of cord-blood-derived or bone-marrow-derived human mast cell cultures have demonstrated the acquisition of chymase staining over time in previously tryptase-positive, chymase-negative mast cells, implying sequential development [92–95]. Another possibility is that immature MC_{TC} cells may produce little, if any, chymase protein, and would

thus resemble MC_T cells until they mature and start expressing chymase protein. Alternatively, MC_{TC} cells may have a survival advantage over MC_T cells in the culture conditions tested, because the latter undergo apoptosis in the presence of IL-4 [96], while skin-derived MC_{TC} cells proliferate better in serum-free medium than do lung-derived MC_T cells [97]. For individuals who have congenital combined immunodeficiency syndromes and patients who have AIDS, marked and selective decreases in MC_T cells occur in the bowel, whereas the concentration and distribution of MC_{TC} cells are unaffected, which suggests that T lymphocyte factors are involved in the normal growth and differentiation of MC_T but not MC_{TC} cells [98].

Ocular mast cells

The normal eye

Several studies have attempted to characterize the number, distribution, and phenotypes of mast cells in the normal eye of various species. As early as 1937, Jorpes and colleagues [99] noted mast cells in the limbal area of normal human eyes. Later, Feeney and Hogan [100] described the presence of mast cells among the nonpigmented cells of the normal human choroid stroma. Using flat whole mounts of the entire uvea stained with toluidine blue at pH 1.6, large numbers of mast cells could be detected in the posterior choroid of the eyes of guinea pigs, rabbits, and rats [101]. The mast cells often were seen lining the external walls of branching arterial vessels. In guinea pigs, concentration of mast cells in the posterior choroid averaged 250/mm² compared with 2 to 12 mast cells/mm² in the intestinal mesentery and the kidney capsule, respectively. In contrast, the iris and ciliary body contained very few, if any, mast cells. Similarly, very few mast cells were identified in the sclera, retina, and cornea. A different pattern of distribution was noted in ferrets and dogs, where few mast cells were seen in the choroid, and most intraocular mast cells were found in the anterior uvea of dogs and in the deep part of the ciliary body in ferrets [102]. Rat choroidal mast cells released histamine when exposed to compound 48/80, indicating properties similar to those of connective tissue mast cells [103]. In humans, most studies of ocular mast cells have been concentrated on the conjunctival tissue. In normal subjects, few, if any, mast cells were found in the conjunctival epithelium, and they appeared to be of the MC_T type. The substantia propria of the normal conjunctiva contained large numbers of mast cells with a concentration of 11,054/mm³; 95% of which were of the MC_{TC} type (Fig. 1A) [104]. Allansmith [105] estimated the number of mast cells in the ocular and adnexal tissues of one human eye at 50 million, and confirmed the virtual absence of mast cells from the normal conjunctival epithelium, cornea, iris, retina, and optic nerve. Mast cells were found scattered in the meninges of optic nerves, usually in perivascular locations.

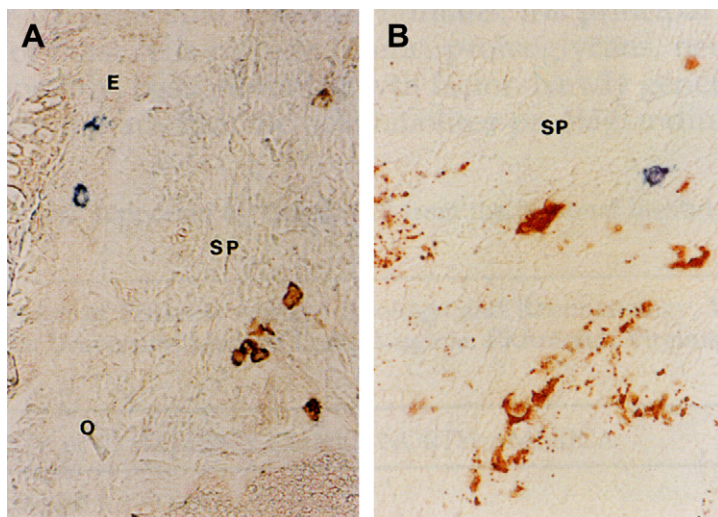


Fig. 1. Sections of human conjunctiva stained by double immunohistochemistry with anti-tryptase and anti-chymase antibodies. MC_T cells are visualized in blue. MC_{TC} cells are visualized in brown. (A) Section of normal conjunctiva showing two MC_T cells in the epithelium and several MC_{TC} cells in the substantia propria (original magnification $\times 1100$). (B) Section of vernal conjunctivitis showing one intact MC_T cell, one intact MC_{TC} cell, and numerous free MC_{TC} granules in the substantia propria (original magnification $\times 1700$). E, epithelium; SP, substantia propria.

Mast cells in diseases of the eye

The eye is a target organ for many pathologic reactions involving immune reactions. Mast cell involvement has been postulated in a variety of ocular disorders, including those involving IgE-mediated reactions and chronic inflammatory disorders of the conjunctiva or the uveal tract.

Allergic conjunctivitis

Direct exposure of the ocular mucosal surface to environmental allergens (and with the large number of conjunctival mast cells), results in the frequent occurrence of immediate hypersensitivity reactions affecting the conjunctiva in atopic subjects. Mast cell activation in allergic conjunctivitis is evidenced by an increase in tryptase levels in unstimulated tear fluid of subjects who have symptomatic allergic conjunctivitis [6]. Similarly, conjunctival provocation of atopic individuals who have relevant allergens results in the release of histamine, kinins, PGD, and TAME-esterase activity in tears [106]. The total number of conjunctival mast cells found in the substantia propria in subjects who have allergic conjunctivitis has been reported to increase mildly ($15,389/\text{mm}^3$) compared with normal control subjects [104]. The number of epithelial conjunctival mast cells is increased, which is similar to findings in the nasal mucosa of subjects who have allergic rhinitis [107,108]. A marked

increase in subepithelial mast cell numbers and epithelial invasion with mast cells has been reported in atopic keratoconjunctivitis as well [109,110]. Whether the epithelial mast cells seen in these conditions represent an increase in precursor mast cells differentiating locally or a migration of subepithelial mast cells into the epithelial layer is not known. However, the characterization of epithelial mast cells as tryptase-positive only (MC_T cells) would argue toward the former theory, because subepithelial mast cells are mostly of the MC_{TC} type. The increase in lymphocytic infiltrate seen in the epithelium of subjects who have atopic keratoconjunctivitis may provide the necessary local environmental factors for differentiation of mast cells toward the MC_T type. Sodium cromoglycate had been reported to cause partial inhibition of degranulation of lung mast cells (mostly MC_T cells), whereas skin mast cells (MC_{TC} cells) appeared resistant to its effects. Therefore, the clinical response seen in subjects who had allergic conjunctivitis treated with topical sodium cromoglycate may result from inhibition of degranulation of conjunctival MC_T cells found in the epithelium.

Vernal conjunctivitis

Increased concentration of mast cells in the conjunctival substantia propria of subjects with vernal conjunctivitis has been reported at the electron microscopy level as well as by immunohistochemistry [111]. Mast cell concentrations averaged 24,689/mm³ and reached an upper limit of 60,632/mm³ in one subject, similar to mast cell concentrations in cutaneous mastocytosis. Furthermore, a significant number of MC_T cells are seen in the epithelium and subepithelium of the bulbar conjunctiva, although MC_{TC} remains the predominant type [104]. The presence of MC_T cells in the inflamed conjunctiva of subjects who have vernal conjunctivitis may reflect permissive local environmental factors, because large numbers of CD4-positive T lymphocytes are known to infiltrate this tissue. Extensive degranulation of mast cells is apparent at the electron microscopy level and by light microscopy, where large numbers of free granules scattered around the substantia propria were observed (Fig. 1B). These findings are consistent with previous results showing elevated histamine and tryptase levels in tears [110,112]. It is unclear if mast degranulation is a primary event in vernal conjunctivitis or if it is a result of the severe eye rubbing associated with this condition, because eye rubbing has been demonstrated to lead to an increase in tryptase level in tear fluid. Treatment of vernal conjunctivitis with cyclosporine eye drops does not alter the numbers, phenotype, or fragmented appearance of mast cells in the conjunctiva (K. Tabbara and Anne-Marie Irani, MD, personal communication, 1995).

Giant papillary conjunctivitis

Contrary to findings in vernal conjunctivitis, mast cell hyperplasia appears to be minimal in giant papillary conjunctivitis, with concentrations of mast

cells averaging $17,313/\text{mm}^3$ [104]. Epithelial mast cells were seen in four out of six subjects studied and were exclusively MC_{TC} cells. A recent report in prosthesis-associated giant papillary conjunctivitis also demonstrated epithelial mast cells in 5 of 17 specimens, but none were demonstrated in normal conjunctiva [113]. In asymptomatic soft contact lens wearers, the concentration and protease phenotype of conjunctival mast cells were similar to normal controls, and no epithelial mast cells were found. Thus, the distribution of MC_{T} and MC_{TC} cells may contribute to the distinct clinical presentations and have important implications regarding the pathogenesis and treatment of these ocular disorders.

Experimental autoimmune uveitis

In this animal model of human autoimmune ocular diseases, an intense bilateral panophthalmic inflammation develops 10 to 18 days after inoculation with the retinal S-antigen emulsified in an appropriate adjuvant, or 3 days after adoptive transfer with a uveitogenic T-lymphocyte line. Several studies have documented an association between baseline mast cell numbers in the anterior uvea and choroid, and the susceptibility to experimental autoimmune uveitis (EAU) [111,114,115]. Two inbred rat strains, CAR and Lewis, are high responders to the induction of EAU, and essentially all animals develop the disorders postimmunization with retinal antigens. On the other hand, only one fourth of Brown Norway (BN) rats and one half of F_1 hybrids of Lewis and BN rats (LBNF) developed EAU. The corresponding mast cell measurement revealed significant numbers of mast cells in the iris, ciliary body, and choroid of Lewis rats and CAR rats, revealed almost absent mast cells in these locations in the BN strain of rats, and revealed intermediate numbers in the LBNF hybrids. These patterns of susceptibility to EAU are identical to the patterns of susceptibility to experimental autoimmune encephalitis (EAE) in the same animal strains. Mice with a genetic mutation in the c-kit tyrosine kinase receptor resulting in mast cell deficiency are resistant to induction of EAU and exhibit delayed onset and decreased severity of the disease as compared with wild-type littermates [116]. Furthermore, reconstitution of mast cell development following bone marrow transplant from syngeneic wild-type animals restores susceptibility to EAE, indicating an important role for mast cells in the disease pathogenesis.

The evidence pointing to mast cell degranulation in EAU is weaker and consists of demonstrating a variable degree of decreasing mast cell numbers in the anterior uvea and choroid following immunization with the retinal antigen, and before or immediately following the development of clinical disease. Mediator levels were measured in tissue homogenates in only one study, and they showed a decrease from control values in the anterior portion of the eye, concomitant with the decrease in mast cell numbers. Histamine level in aliquots of aqueous fluid from the eyes of EAU rats

did not vary significantly from those of saline controls [111]. These results would argue against the occurrence of mast cell degranulation and in favor of apoptosis of mast cells or inhibition of mast cell differentiation. Histamine levels were reported to be increased in the choroid and the retina.

Summary

Mast cells are resident cells in the ocular and adnexal tissues of the normal eye and are concentrated in the conjunctival subepithelium, posterior choroid, and along limbal vessels. In normal conjunctiva, the majority of mast cells are of the MC_{TC} type. The cornea, iris, retina, and optic nerve are devoid of mast cells in humans. Species difference in the distribution of ocular mast cells may account for differing disease susceptibility to autoimmune phenomenon. The role of mast cells in ocular allergy is well established, as it is in allergic disorders involving other organs, such as the nose, lungs, and skin. In addition, involvement of mast cells in chronic inflammatory disorders of the eye, such as vernal conjunctivitis, giant papillary conjunctivitis, and atopic keratoconjunctivitis, is suggested by epithelial invasion of the conjunctiva with mast cells by an altered mast cell phenotype and by the release of mast cell mediators in tear fluid. Advances in the understanding of mast cell biology in general, and of mast cell involvement in ocular disorders in particular, should lead to the development of new therapeutic approaches based on the specific immunopathology and the selective participation of different types of mast cells.

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