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Article type : Review

Clinical implications of mast cell involvement in allergic conjunctivitis

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Keywords Allergic conjunctivitis; Conjunctiva; IgE; Mast cell; Mediators

Abbreviations:

AC Allergic Conjunctivitis

Ag Antigen

AKC Atopic Keratoconjunctivitis

BMMCs Bone marrow derived mast cells

DCs Dendritic Cells

ECP Eosinophil cationic protein

LTs Leukotrienes

MCs Mast Cells

PAC Perennial Allergic conjunctivitis

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13334

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PGs Prostaglandins

SAC Seasonal Allergic Conjunctivitis

VKC Vernal Keratoconjunctivitis

MIP-1 α (CCL3) Macrophage inflammatory protein 1 α

MCP-3 (CCL7) Monocyte chemoattractant protein-3

VCAM-1 Vascular cell adhesion molecule 1

VLA-4 Very Late Antigen-4

Short title: Role of mast cells in allergic conjunctivitis

Abstract

The conjunctiva is a common site for the allergic inflammatory response due to it being highly vascularized, having constant exposure to environmental pollutants and allergenic pollens and having a unique conjunctival associated lymphoid tissue. The primary morbidity of anterior surface conjunctival disorders that include allergic conjunctivitis and tear film disorders is associated with its high frequency of involvement rather than its severity, although the more chronic forms can involve the cornea and lead to sight threatening conditions. Ocular allergy is associated with IgE-mediated mast cell activation in conjunctival tissue leading to the release of preformed mediators including histamine and proteases and subsequent de novo formation of lipid-derived mediators and cytokines that trigger a cascade of cellular and molecular events leading to extensive migration and infiltration of inflammatory cells to the ocular surface. The trafficking of neutrophils, eosinophils, and lymphocytes to the ocular surface is due to establishing various chemokine gradients (mainly CCL11, CCL24, CCL5, MCP-3, and MCP-4), cell surface expression of adhesion molecules (such as VCAM-1 the ligand for VLA-4), and leukocyte adhesion to vascular endothelium. The release of preformed mediators underlies the acute ocular surface response while the secondary influx of inflammatory cells leading to the recruitment and activation of eosinophils and the subsequent activation of Th2 and Th1 lymphocytes at the level of the conjunctiva reflects the late-phase reaction.

Introduction

The eye is a potential target of allergic inflammatory disorders because of its marked vascularity, sensitivity of conjunctival vessels and direct contact with the environment owing to its anatomical position; thus, to interact with offending antigens it benefits from a complete array of immune cells and related molecular mechanisms (1). Allergic conjunctivitis (AC) is a collection of heterogeneous ocular inflammatory conditions mediated by mast cell activation, affecting the conjunctiva, eyelids and cornea (2). AC commonly co-exists with other allergic diseases including asthma, atopic dermatitis, and rhinitis (3). Allergic eye disorders are primarily mediated by the interaction of IgE and mast cells (MC) that progress to involve T lymphocytes (4). The allergic events during AC occur following exposure to allergens mainly pollens, animal dander, and mold spores. Mast cell activation takes place in two phases: an acute phase, occurring within minutes, and a late phase, spanning from 6 to 72h after exposure. The acute-phase reaction is due to the release of preformed (e.g. histamine) and inducible mediators after the cross-linking of allergen-specific IgE on the surface of resident MCs (5, 6). The late-phase reaction is characterized by the release of chemokines including CCL11, CCL24, CCL5, monocyte chemoattractant protein-3 (MCP-3,CCL7), CCL13, and macrophage inflammatory protein 1 α (MIP-1 α ,CCL3) and the infiltration of eosinophils, basophils, T cells, neutrophils and macrophages that generate a spectrum of other mediators adding to conjunctival inflammation (5). (Figure1A) In addition to histamine, tryptase and other proteases, arachidonic acid metabolites (leukotrienes, prostaglandins),

cytokines, chemokines, growth factors, and adhesion molecules participate in the pathogenesis of the allergic inflammation of the ocular surface (7). Acute ocular allergy symptoms include itching, tearing and swelling and with unchecked ongoing chronic ocular inflammation from eosinophils and Th2-generated cytokines on the ocular surface there is increase potential impact on the visual axis (4, 8). The tear film that blankets the ocular surface improves refraction, lubrication, nutrient delivery, and protection to the epithelial surface. The tear fluid contains specific inflammatory markers including histamine, tryptase, eosinophil cationic protein (ECP), IL-4, IL-5 and eotaxin as well as IL-1 β , IL-6, IFN- γ , and TNF- α . Studies of cytokines have provided a distinctive insight into the pathogenesis of anterior surface disorders of the ocular surface such as ocular allergy and tear film dysfunction (dry eye syndromes)(9, 10).

Conjunctival cells involved in type I hypersensitivity

The conjunctiva is one of the most common sites of allergic inflammation because its mucosal surfaces are highly accessible to airborne allergens. Initial ocular exposure of a genetically predisposed individual to a novel allergen occurs during the sensitization phase. The allergens are processed and presented by CD11b⁺ or CD103⁺ dendritic cells and/or other APCs including CD11b⁺ and CD68⁺ macrophages located in the mucosal epithelium of the conjunctiva and the limbus. The interaction between these APCs and naïve CD4, or T helper (Th0) cells leads to their maturation and differentiation into effector Th1 or Th2 lymphocytes. Th2 cells are mainly involved in IgE mediated allergic response through releasing IL-3, IL-4, IL-5, IL-9, IL-10 and IL-13 which contribute in allergic events including IgE production by B cells, mast cell growth, eosinophil accumulation and increased mucus production (11). Sensitization and differentiation of Th2 cells requires Ag presentation by DCs. Ocular surface DCs contribute to the immunopathogenesis of AC via upregulation of CCR7 in response to allergens and response chemotactically to a CCL21 gradient established by local lymphatic endothelial cells. Indeed, CCR7-CCL19/21 chemokine axis has been reported to have a key role in trafficking of mature DCs to eye-draining lymph nodes where they interact with T cells (12). B lymphocytes upon activation by BCR-(MHCII-peptide) interactions proliferate and differentiate into plasma cells, and secrete antigen-specific IgE that binds to its high-affinity receptor IgE receptor (Fc ϵ RI) located on the surface of MC and basophils (11).

MCs contribute to recruitment of inflammatory cells to conjunctiva

MCs upon activation and degranulation release a wide range of mediators including TNF α and IL-4 (13). TNF α stimulates the transcription of chemokines in conjunctival fibroblasts and epithelial cells to release IL-8 (acts through CXCR1 and CXCR2 receptors) and CCL5 which are capable of recruiting lymphocytes and neutrophils. CCL5 acts through CCR1, CCR3, CCR4 and CCR5 as a non-selective chemokine for various inflammatory cells. IL-4 induces the release of CCL11 from fibroblasts which acts through CCR3 to recruit eosinophils. Recruited inflammatory cells induce tissue damage by producing a variety of cytotoxic substances, including eosinophil cationic protein (ECP), eosinophil peroxidase, neutrophil toxic oxygen radical, proteases and Th2 lymphocytic inflammatory cytokines (14). (Figure1B) Interestingly it has been reported that the expression of HSP-70 and PAR-2 increases during AC which is associated with an increase in conjunctival epithelial thickness. Moreover, PAR-2 expression on epithelial cells alters epithelial permeability by the disruption of epithelial E-cadherin. Thus, increased expression of PAR-2 in SAC patients exposed to pollen facilitates antigens entering to the subepithelial space to activate MCs (11, 15).

Epithelial cells of the conjunctiva have been shown to express IL-33 constitutively. It is also reported that IL-33 levels increase during ocular allergy and that IL-33 induces mouse basophils and MCs, which express IL-33R abundantly to produce cytokines including IL-4, IL-6, IL-9, IL-13, GM-CSF and chemokines (CCL5, MIP-1 α , MIP-1 β and MCP-1). It also stimulates antigen-stimulated Th2 cells to strongly increase production of IL-4, IL-5 and IL-13 (16). The severity of AC correlates with chemokines/adhesion molecules dependent eosinophil infiltration into the conjunctiva. Fukushima et al. reported that VLA-4 expressing inflammatory cells mainly eosinophils and lymphocytes infiltrate into the conjunctiva via interacting with vascular cell adhesion molecule (VCAM)-1 (CD106) the ligand of VLA-4 and fibronectin(17).

Human conjunctival MCs express various chemokine receptors, including CCR1, CCR2, CCR3, CCR5, and CXCR3 through which signaling results in MC activation or recruitment of other immune cells (18, 19); for instance, MIP-1 α - CCR1 interaction is required for the immediate hypersensitivity reaction and MC activation and CCL11- CCR3 interaction, is needed for activation and homing signal for eosinophils (18). Experimental allergic conjunctivitis initiated in wild-type and CCR3 $-/-$ mice by passive transfer of ragweed (RW)-specific IgE, followed by topical challenge with RW in eye drops showed that CCR3 is associated with both the early-phase reaction and late-phase inflammation during ocular allergy (20). Ag-specific TH2 cells contribute to conjunctival eosinophil infiltration in severe types of AC such as vernal keratoconjunctivitis (21). Moreover, it is hypothesized that infiltrating antigen-specific IL-4 producing Th2 cells activate fibroblasts to release eosinophil-attracting chemokines, including eotaxin (17). CD4+, CD45+RO and HLA-DR+ T cells have also been reported in conjunctiva of patients with atopic and vernal keratoconjunctivitis (22). (Figure 2)

Types of anterior ocular allergies

The symptoms and signs observed in AC are the end result of multiple factors, including genetics, environment, ocular surface microbiome and immune regulation (2). Classification of different types of allergic conjunctivitis has been controversial. However, according to the clinical manifestations, course, and prognosis differences, allergic conjunctivitis is divided into types listed in table 1. La Rosa et al. proposed a new classification that aims to distinguish the pathophysiologic mechanism of the disease and suggested that SAC and PAC (as well as intermittent and perennial forms) be combined under the name of “allergic conjunctivitis” while the new term of “multifactorial conjunctivitis” include VKC and AKC. Finally, GPC remains, as a newly classified traumatic disorder (23).

The diagnosis of ocular allergy is primarily clinical, however, there are laboratory tests used for supporting the diagnosis (24). Skin testing for specific allergens by epicutaneous (scratch/prick) or intradermal injections of possible allergen as well as conjunctival provocation tests provide in vivo evidence of specific IgE sensitization. In-vitro tests for IgE antibodies to specific allergens also exist. Additionally, commercial tests for total IgE levels in tears are available, but have limited use as the spectrum of total IgE results does not appear to differentiate the clinical allergic disorders of the anterior surface of the eye. Conjunctival smears can provide supportive evidence of AC as the presence of eosinophils is supportive of an allergic diathesis (25).

Infectious conjunctivitis caused by viral and bacterial pathogens (mainly staphylococci bacteria and adenoviruses) may be distinguished from allergic disease by conducting a thorough history and physical examination since the former type typically causes ocular burning, foreign body sensation, stinging, discomfort with a “gummy” discharge commonly causing a glue eye, rather than itching (26). The key

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symptoms in bacterial conjunctivitis include soreness of the eye and redness that is typically unilateral, with purulent discharge while key symptoms observed in viral conjunctivitis include mild discomfort typically bilaterally with watery and mucoid discharge (27). Conjunctival swabs for the culture or polymerase chain reaction techniques may show infectious etiology in Infectious conjunctivitis. Additionally, conjunctival cytology, if available, could be a practical tool in differential diagnosis of ocular allergies.

Follicular conjunctivitis is a common differential diagnostic eye disease that is asymptomatic or mild and may not require treatment (3). Another pathologic condition which may cause damage to the conjunctiva and corneal epithelium and predispose conjunctiva to infection is Sjögren's syndrome. The disease is an autoimmune disorder characterized by reduced lacrimal and salivary gland function and leads to the sicca complex (dry eyes and dry mouth) (27). Remodeling and/or structural destruction of the basement membrane scaffolding in salivary glands of these patients is attributed to matrix metalloproteinase-9 (MMP9). Since MC-derived chymase can cause breakdown of extracellular matrix (ECM) and activation of MMP9 precursor, mast cells may play a role in pathogenesis of Sjögren's syndrome (28, 29).

Conjunctival mast cells activation and intracellular signaling

MCs express FcεRI and cross-linking of IgE /FcεRI complex with the allergen based epitope activates mast cells to release their preformed mediators (e.g. histamine, proteolytic enzymes and proteoglycans) as part of the early-phase response that occurs within minutes, and subsequently rapidly synthesizes lipid mediators (e.g. leukotrienes and prostaglandins) as part of the late phase response(30). The cross-linking induces the phosphorylation of ITAM motifs in the FcεRI β and γ chains which results in activation of Syk by Lyn. Consequently, phosphorylation of several tyrosine residues in LAT and activation of adaptor molecules occur. Syk and Btk activate PLCγ, the enzyme which catalyzes the production of IP3 and DAG (diacylglycerol), leading to Ca²⁺ mobilization and the activation of PKC isoforms. Together, signaling through several pathways results in activation of transcription factors such as of the NFAT and activator protein-1 gene families. The final outcome of signaling and gene activation is production and release of a wide spectrum of MC derived mediators (31-33) including IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, GM-CSF, MIP-1α, and TNF-α. Among them, IL-3 and IL-5 contribute to eosinophilic inflammation since they are involved in eosinophil development, survival, and recruitment (34).

Subtypes of conjunctival MC

One of the primary studies clarifying the role of human MCs in pathogenesis of allergic conjunctivitis was completed by Henriquez et al (1981). They studied the granules of conjunctival MCs from normal individuals and those with vernal conjunctivitis by electron microscopy and observed that most MCs from normal subjects were fully granulated while MCs of patients with vernal conjunctivitis were found to be extensively degranulated (35). Moreover, Irani et al (1990), studied the distribution of MC subpopulations in human conjunctiva using a double immunohistochemical method benefitting from murine monoclonal immunoperoxidase conjugated anti-chymase antibody followed by alkaline phosphatase conjugated murine monoclonal anti-tryptase antibody demonstrating that MC_{TCs} are the predominant type in the human substantia propria. An increase in the number of MC_{TS} was observed in

the conjunctiva of subjects with vernal conjunctivitis, but not in subjects with giant papillary conjunctivitis (GPC) or allergic conjunctivitis (AC)(36). In VKC, MCs have been reported to express TLR-4, TLR-6, and TLR-8, but not TLR-5. Furthermore, activation of TLR-2 and TLR-4 induces MC degranulation and release of Th2 cytokines (37).

Role of mast cell cytokines/ mediators in allergic conjunctivitis

The activation of conjunctival MCs provides a cascade of events that leads to the allergic inflammatory response of the ocular surface. Cytokines include the expression of IL-4 and TNF α that promote eotaxin and ICAM-1 (CD54) and Kit expression and secretion from corneal epithelial cells, keratocytes and conjunctival fibroblasts (38, 39). The activated fibroblasts then provide additional proinflammatory cytokines and chemokines to the tear film bathing the conjunctival surface with CCL5, monocyte chemoattractant protein (MCP), IL-8, eotaxin, and macrophage inflammatory protein 1 α (MIP-1 α) that can be found in tear film obtained from SAC, PAC, AKC, and VKC patients (40). IgE-Fc ϵ RI cross-linking generates a signal for cleavage of mast cell membrane phospholipids leading to release of phospholipase A2 (PLA2), prostaglandin D2 (PGD2), leukotrienes B4, C4, and D4 (LTB4, LTC4, LTD4), and platelet-activating factor (PAF). Prostaglandins are associated with an increase in microvascular permeability and augments histaminic effects of ocular surface hyperemia, pruritus, and mucus hypersecretion through stimulating the goblet cell (41). Additionally, preformed mediators predominantly are stored in mast cell granules (e.g. histamine, proteoglycans, tryptase, neutral serine proteases, chymase and carboxypeptidase) (38). Chymase is a potent converter of angiotensin I to angiotensin II and the IL-1 β precursor to its active form (42). Moreover, it has been reported as a pharmacotherapeutic target for AC in animal model (43). In an allergic conjunctivitis animal model, the application of a chymase inhibitor (ONO-WH-236), in a dose dependent fashion inhibited conjunctival edema and erythema (42). Classically, the histamine H1 receptor has been the target of therapy for AC, but the H2 receptor is also involved in the development of vasodilation of the ocular surface. Histamine binding to specific receptors results in increased release of IL-1 β , IL-6, IL-8, IL-10, IL-11, eotaxin and GM-CSF (32). The contribution of H4-receptor in itching has been investigated using knockout mice that demonstrated fewer bouts of scratching when they were given a dose of histamine in comparison to wild-type animals. H4 receptors are expressed in leukocytes, MCs and peripheral hematopoietic cells and their stimulation leads to MC activation, eosinophil recruitment and differentiation of myelocytes and promyelocytes (44).

Animal models of allergic conjunctivitis

We owe most of our knowledge about AC to murine model experiments. Typically, the protocols include comparing the symptoms induced by an allergen in two groups of wild type (WT) and MC-deficient or chemokine/mediator gene-deficient mice. The final step of such protocols commonly includes the reconstitution of tested mice with MCs from WT mice and observing the restoration of symptoms of AC. Here we review two studies using mice model in their protocols. Fukuda et al in order to determine the precise role of conjunctival MCs during type I ocular allergy managed to reconstitute conjunctival MCs of Kit^{W-sh/W-sh} mice through adoptive transfer of Kit^{+/+} bone marrow-derived, cultured mast cells (BMCMCs) from congenic wild-type C57BL/6J-Kit^{+/+} mice. Six to eight weeks after subconjunctival transfer of BMCMCs, mice displayed significant local repair of MC populations in both the conjunctival substantia propria and the eyelid dermis. Passive transfer of ragweed-specific IgE following by antigen challenge resulted in both early-phase clinical symptoms and late-phase eosinophilic inflammation in Kit^{+/+} mice while both types of responses were significantly decreased in Kit^{W-sh/W-sh} mice. They also applied

trinitrophenyl (TNP)–keyhole limpet hemocyanin–specific IgE for passive sensitization for mice from each group and compared the responses observed in mice passively sensitized by RW specific IgE to confirm that responses are IgE specific. Reconstitution of conjunctival MCs could successfully restore early and late-phase responses in conjunctival MC reconstituted $\text{Kit}^{\text{W-sh/W-sh}}$ mice (45). (Figure 3). Miyazaki et al, provided a line of evidence by investigating the early and late inflammatory responses in $\text{Kit}^{\text{W}}/\text{Kit}^{\text{W-v}}$ mice and conjunctival MC reconstituted $\text{Kit}^{\text{W}}/\text{Kit}^{\text{W-v}}$ mice. This group of researchers used a quite different protocol for obtaining conjunctival MCs to be used for adoptive transfer. Unlike other studies using mice bone marrow mast cells (BMMCs) to reconstitute the MC population of $\text{Kit}^{\text{W}}/\text{Kit}^{\text{W-v}}$ mice, they managed to reconstitute the conjunctival MCs of deficient mice with conjunctival MCs from wild type mice. Indeed, BMMCs were not appropriate in conjunctival MC investigations due to different profile of marker expression. For example, they do not express CCR3 which is an important maturation marker of connective tissue type MC. To assess the success of the transfer process the conjunctival expression of mMCP-5 and mMCP-6 was evaluated since they are not expressed in the conjunctiva of $\text{Kit}^{\text{W}}/\text{Kit}^{\text{W-v}}$ mice. For the purpose of immunization and induction of experimental allergic conjunctivitis they used single and multiple allergen exposures. Single exposure was done by injecting ragweed pollen plus aluminum hydroxide in both types of mice. Conjunctivitis was induced by topical application of ragweed suspended in phosphate buffered saline (PBS). On day 22 symptoms including conjunctival edema, lid edema, tear discharge, and conjunctival redness were graded. It was revealed that the early phase and eosinophilic responses were minimal in $\text{Kit}^{\text{W}}/\text{Kit}^{\text{W-v}}$ mice after sensitizing but were fully restored following adoptive transfer (34). Fukuda et al, using a CCR3-/- mice model investigated the role of CCR3 (expressed on eosinophils and MCs) in both the early-phase reaction and late-phase inflammation during ocular allergy following RW challenge. They reported no significant difference in number of conjunctival MCs between CCR3-/- mice and WT mice (20).

Treatment of allergic conjunctivitis

Treatment of AC includes allergen avoidance, pharmacotherapy and allergen-specific immunotherapy (SIT). Table 2 contains the main groups of drugs being used in AC pharmacotherapy and their main features and clinical application.

Antihistamines

Histamine receptors are primary topical drug targets in AC since interaction between histamine and its receptors are primarily responsible for signs and symptoms. Four histamine receptors H1-H4 have been discovered. H1 and H2 receptors are linked to pruritus, conjunctival hyperemia, cytokine secretion, fibroblast proliferation, adhesion molecule expression, microvascular permeability and production of procollagens (2). Blocking the action of histamine alone does not abrogate the entire allergic response, since it is released along with other inflammatory indicators, including PAF, eosinophil chemotactic factors and prostaglandins. Thus, the focus for novel agents in the treatment of anterior ocular allergic disorders has been on combining histamine H1 receptor antagonism with other mechanisms including the stabilization of MC membranes and anticytokine activity (46). Antazoline (ANT) is the first-generation antihistamine that represents a classic histamine H1 receptor inverse agonist that generates a reversible competition effect on histamine receptor. As a first-generation antihistamine, Antazoline possesses anticholinergic properties and has been employed in combination with decongestants such as

naphazoline and/or tetrahydrozoline (TET) to reduce the symptoms of allergic conjunctivitis. Its mechanisms of action include constriction of conjunctival blood vessels (47). Long-term use of the antihistamine antazoline and the vasoconstrictor naphazoline is not recommended because they both can cause rebound hyperemia (48). Levocabastine hydrochloride is a selective histamine H₁-receptor antihistamine which is used worldwide as a topical treatment for AC and allergic rhinitis (49). Preservative-free levocabastine 0.05 % ophthalmic solution has been reported effective in the prevention of allergic conjunctivitis induced by a conjunctival provocation test (50).

Mast cell (MC) stabilizers

MC stabilizers such as cromolyn have been used for the treatment of mild and moderate symptoms of AC, but in the United States are indicated for the treatment of more severe chronic forms of ocular allergy having pannus formation or corneal ulcers associated with VKC and GPC. Interestingly, they have also been reported to improve nasal symptoms due to direct drainage onto the nasal mucosa through the nasolacrimal duct (51). For optimum symptomatic relief, these group of agents need to be administered prior to exposure and may require up to 6 weeks to gain their full effect (52). Nedocromil sodium, a pyranoquinoline dicarboxylic acid, is an anti-inflammatory drug that had the unique effect of causing yellow stains on clothing, but similar to other cromolyn based agents requires multiple daily doses for maximum effect (53). Lodoxamide, another mast cell stabilizer was reported to be approximately 2500 times more potent than cromolyn in the prevention of histamine release in animal models requiring multiple doses for effect (54). It is the most effective drug for controlling the early phase inflammatory process of VKC and alleviating severe vision disturbing signs and symptoms (55).

Dual action agents

Dual action agents group includes drugs such as Olopatadine, Ketotifen, Bepotastine, Alcaftadine and Azelastine which act both as H₁ antihistamines and MC stabilizers. Some of these drugs have multiple action and immunological effects, for instance, Bepotastine may have a role in inhibition of eosinophil migration, IL-5, leukotrienes (e.g., LTB₄) and PAF (56). Additional properties of Alcaftadine include H₂ and H₄ receptor inverse agonism, inhibition of MC histamine release, decreased eosinophil chemotaxis and activation (57). Ketotifen is an H₁ antihistamine that also inhibits release of histamine and inflammatory mediators from MCs and eosinophils. It can prevent the infiltration, activation, and degranulation of eosinophils. Ketotifen is used in different conditions such as asthma, allergic rhinitis in addition to AC (58). Ketotifen, which appears to have multiple actions, is distinguishing itself from other cromolyn compounds (e.g. sodium cromoglycate, nedocromil), by a conjoint mast cell stabilizing, and H₁ receptor inverse agonism (59) as well as inhibition of leukotriene formation (60). Olopatadine hydrochloride is a relatively selective histamine H₁-receptor inverse agonist that possesses mast cell stabilizing features (61). It is available in 3 formulations (0.1%, 0.2% and 0.7%). Olopatadine has demonstrated capable inhibition of various cytokines (62) including TNF α (63). It is active in the conjunctival allergen challenge model and has a rapid onset of action (within 20 minutes after instillation) and a duration of ≥ 8 hours (64). Topical multiple-action agents appear to inhibit the effects of histamine as well as the synthesis of additional inflammatory mediators that accentuate the late-phase response of the anterior surface of the eye (65).

NSAIDs

Cyclooxygenase (COX) enzymes including COX-1 and COX-2 catalyze the conversion of arachidonic acid into prostaglandin (PG) G₂, an unstable intermediate that is rapidly converted to PGH₂. Subsequently, PGH₂ is then metabolized into different structurally-related PGs, mainly PGE₂, PGD₂, PGF₂, PGI₂ and thromboxane (TX)A₂ (66). Topical NSAIDs inhibit COX-1 and COX-2 resulting in inhibition of inflammatory mediators such as prostaglandins and leukotrienes. However, they do not block mediators in the lipoxygenase pathway such as leukotrienes. They successfully alleviate symptoms like pain, irritation and hyperemia, and are approved for the most part for post-operative inflammation (2). Ophthalmic NSAIDs such as ketorolac have demonstrated antipruritic properties through reducing prostaglandin levels in tear film, but may cause a drop in patient compliance because of stinging and burning upon instillation (67). Ketorolac tromethamine was originally formulated as a 0.5% solution marketed in the US and Europe. After reporting burning and stinging upon instillation, a new formulation with a lower concentration, 0.4%, was introduced in the US in 2003 (68). Diclofenac sodium 0.1% became available in US market in 1991 and is approved to treat postoperative inflammation in patients who have undergone cataract extraction and for the temporary relief of pain and photophobia in individuals undergoing corneal refractive surgery (69).

Corticosteroids

Corticosteroids are able to repress the production of several pro-inflammatory cytokines such as IL-1, IL-4, IL-5 and TNF- α as well as the expression of adhesion molecules such as ICAM-1, VCAM-1 and VLA-4 that consequently decrease eosinophil, neutrophil, MCs and lymphocytes infiltration at the site of inflammation (70). Topical corticosteroids are associated with potent ocular effects, including the development of elevated intraocular pressure (IOP) leading to glaucoma, cataracts, and infection. Thus, they are recommended to be utilized over short periods of time (up to 2 weeks) and for use beyond 2 weeks, with IOP and lens clarity being monitored if used for more than 4 weeks (65). The topical steroids have different potencies depending on their corneal penetration. Relative potencies from mildest to strongest effects include loteprednol, rimexolone, fluorometholone, prednisolone (phosphate < acetate) to dexamethasone. Loteprednol has the unique claim of being a 'site-specific' steroid, due to residing at the target tissue long enough to render a therapeutic effect but not long enough to cause the secondary harmful effects of, for example, raised IOP (71, 72). Topical or systemic administered steroids will transiently produce an increase in IOP in susceptible individuals that is thought to be genetically influenced. IOP effects are consistent among the different esters of the same corticosteroid base that are not dependent on their efficacy. In general, steroids with low absorption (e.g. fluorometholone) should be used first with the dosing being based on the state of inflammation of the eye, and only when these are proven to be ineffective, should the more potent steroids (including prednisolone, dexamethasone, betamethasone) be employed (73).

Immunomodulatory agents

The focus of the immunomodulatory agents used in controlling the more severe forms of ocular allergy are due to the inhibition of Th2-cell activation involved in the allergic response through binding to the

intracellular receptor protein cyclophilin-1, which subsequently interferes with the dephosphorylation and activation of the main transcription factor of activated T-cells (NF-AT). Consequently, expression of allergic pro-inflammatory cytokines including IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, TNF α , and interferon- γ are suppressed (74). One of the classic immunomodulatory agents used, Cyclosporin A (CsA), is a fungal metabolite that reduces ocular inflammation by inhibiting Th2 lymphocyte proliferation, interleukin-2 production, and histamine release from MCs and basophils. The CsA cyclophilin complex blocks calmodulin/calcineurin-induced phosphorylation of transcription factors that are involved in early T-cell gene expression such as interleukin-2 (75). Topical CsA, has received attention for its ability to reduce corticosteroid usage and its potential as an alternative treatment for corticosteroid-resistant VKC cases (76). Cyclosporine eye drops, either at 1% or 2% concentrations, have been shown to be safe and effective for long-term treatment of VKC in children (77). Tacrolimus, also known as (FK506), was among the first macrolide immunomodulatory agents that has demonstrated potent in vitro immunosuppressive effects on T cell activation. Tacrolimus 0.03% has been demonstrated to be relatively safe and effective in treating patients with steroid-refractory VKC, while the lower concentration, Tacrolimus 0.1% ointment, has also been reported to be effective treatment for VKC refractory to standard treatment (78). Topical tacrolimus is generally well tolerated. The most common side effect is mild and transient ocular irritation and burning sensation which may occur upon drop instillation, but usually does not necessitate discontinuation of treatment (79). (Figure4)

Discussion and conclusion

The role of mast cells in pathogenesis of allergic conjunctivitis through cell to cell interactions and the release of an extensive array of allergic inflammatory mediators has been known for decades. What makes the study of conjunctival MCs complicated, is the fact that unlike other inflammatory cells that circulate in high concentrations and can be easily isolated from peripheral blood, MCs are not found in circulation and are enzymatically dispersed from the target tissues at comparatively low yields. The routine protocols being used from 80s include incubation of finely chopped tissue with collagenase or a mixture of collagenase/ hyaluronidase (80, 81) or collagenase/elastase (82) in HEPES buffer to release the MCs from tissue. Moreover, no solid human models to study conjunctival MCs and their impact in allergic reactions are available. Unlike animal model experiments, obtaining samples from conjunctival tissue is not regularly practiced, however, there are investigations regarding to mast cell involvement in ocular diseases that benefit from samples from normal human subjects at autopsy, or fetuses aborted for chromosomal abnormalities (83). The cytokine profile of tears has partially compensated such shortage proving a window into the activity of the ocular surface. MCs obtained from mice bone marrow cell culture (BMCC) are typically being used to investigate the role of MCs or their chemokines/mediators in reconstitution of MC-deficient mice and chemokine/mediator gene-deficient mice. However, this model does not appear to be appropriate for ocular residing MCs and BMMCs such as surface molecule expression (connective tissue versus mucosal)(34). The type and concentrations of MC mediators in tears have been partially characterized as evidence to the central role of conjunctival MCs in the development of ocular allergic reaction. Advances beyond the blocking of histamine are starting to surface such as the treatment of AC by antagonizing MIP-1 α interaction with its receptor CCR1 or blocking the signal transduction cascade from CCR1; antagonists of MIP-1 α that have a role as a costimulatory signal for MCs(84); induction of IL-10 (and IL-4) to block c-Kit and Fc ϵ RI expression, and induction of MC apoptosis. Bundoc and Keane-Myers in their in vivo animal model of AC studied

stimulated conjunctival mice MCs with C48/80 and reported that conjunctival MCs of IL-10 knockout mice exhibit increased degranulation compared with C57BL wild type control mice. Obtained data suggest a therapeutic potency of IL-10 in treatment of the disease (85). Endogenous IL-17 is needed for the Ag sensitization in murine allergic asthma as a Th2-mediated disease, however it is reported by Fukushima et al that IL-17 is not significant in the development of experimental murine allergic conjunctivitis (86). Finally, although routine drugs mostly target activation/degranulation of MCs, new molecular approaches have been suggested to treat AC, for instance CD11b and F4/80 have been reported to upregulate on conjunctival infiltrating eosinophils. Systemic anti-CD11b and anti-F4/80 Ab treatments during the effector phase could suppress their infiltration in ragweed-induced conjunctivitis (87). *In vitro* and *in vivo* suppression of human MCs and reducing their numbers in patients using imatinib and other KIT-targeting tyrosine kinase inhibitors appears promising in the treatment of allergic disorders including allergic conjunctivitis in humans (88). Considering the molecular pathways by which MCs contribute to recruitment of inflammatory cells to the conjunctiva, their mediator potency to activate other cells and successful targeting of the release of mediators as the main treatment approach in AC, MCs remain the key target in the control of allergic inflammation of the ocular surface.

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Table1: Classification of AC types according to clinical manifestations, course, and prognosis differences

	Main features	Ref
Seasonal Allergic Conjunctivitis (SAC)	Also called “hay fever” conjunctivitis, seasonal rhinoconjunctivitis or conjunctivorhinitis which are the most common form of disease. The onset coincides with regional seasonal increase in circulating allergens (tree pollens in early spring; grasses in May through July; and weed pollens and outdoor molds from August through October). Generally considered having mild to moderate morbidity, but it is not sight-threatening. Ocular pruritus is the predominate symptom with conjunctival hyperemia and with more chronicity of condition papillae in tarsal conjunctiva are observed.	(89) (7) (4)
Perennial Allergic Conjunctivitis (PAC)	Persistent IgE mediated degranulation of MCs prompts the recruitment of eosinophils. Patients experience symptoms throughout the year (main difference with SAC). The symptoms arise from singular and/or multiple indoor allergens, such as animal dander, molds, and dust mites. PAC differs from SAC in that the persistent IgE mediated degranulation of MCs prompts the recruitment of eosinophils, moreover, PAC tends towards chronicity because of the continued exposure to allergen throughout the year.	(7)
Vernal Keratoconjunctivitis (VKC)	VKC mainly affects children and young adolescents, often complicated by keratitis and corneal ulcers main symptoms include intense itching and photophobia. Signs include redness, lid swelling, inflammatory ptosis, ropy discharge, and eosinophilic granulomas associated with severe corneal involvement and visual loss. T cells and eosinophils predominate with a Th2 cytokine-driven inflammation. It is particularly characterized by T cell-mediated responses and increased concentrations of conjunctival T cells. Cytotoxic proteins released from eosinophils infiltrating locally into the conjunctiva are thought to cause keratoconjunctival disorders such as severe AKC and VKC. The hallmark that differentiates VKC from other allergic conjunctivitis is the presence of limbal and giant cobblestone papillae on the upper tarsal conjunctiva. Corneal neovascularization with extensions of limbal vegetation affecting vision, shield ulcers, pseudogerontoxon, and keratoconus may occur in severe cases.	(3) (90) (91) (7) (92) (93)

Atopic Keratoconjunctivitis (AKC)	It is bilateral chronic inflammatory disease of the ocular surface and eyelid. Pathomechanism involves both chronic IgE mediated MC degranulation and immune mechanisms mediated by Th1- and Th2-lymphocyte derived cytokines. Eczematous lesions may be found on the eyelids. The disease is associated with severe corneal involvement and visual loss.	(24) (90)
Giant Papillary Conjunctivitis (GPC)	GPC is not considered as a true allergic condition but rather a chronic inflammatory disease. Hypersensitivity reaction typically caused by contact lens wear. It could be associated with ocular prostheses, postoperative sutures. GPC is characterized by a T cell-mediated response.	(94) (7)

Table2: Main groups of drugs being used in AC pharmacotherapy and their main features

	Main examples	Main feature and clinical application	Ref
Antihistamines	Antazoline Pheniramine, Levocabastine Emedastine	Mechanism of action includes Competitive blockage of histamine receptors (all block H1, and some block H2, H3 and/or H4). antihistamines cause drying of the ocular surface.	(2) (95)
Mast cell stabilizers	Cromolyn Nedocromil sodium Pemirolast Lodoxamide	They inhibit MC degranulation and release of histamine. Discovery of MC heterogeneity abdicated relevance of most of them in AC. Cromolyn proven not active in human conjunctival MC.	(2)
Dual action agents	Ketotifen, Olopatadine, Azelastine Epinastine	They have a rapid onset of action and have a safe side-effect profile. Act as inverse agonists of H1 receptor and prevent MC degranulation. They reduce both the early and late phases of allergic ocular inflammation.	(96) (97) (94) (98)

Corticosteroids	Hydrocortisone, Triamcinolone, Clobetasone butyrate, Fluomethalone, Rimexalone, Prednisolone, Dexamethasone Loteprednol	Corticosteroids avoid releasing prostaglandins and other inflammatory substances. Side effects include increasing intraocular pressure and risk of cataract formation.	(4)
Immunomodulatory agents	Cyclosporine A Tacrolimus	Mainly used in transplantation medicine. They are used for the treatment of severe VKC and AKC and dry eye disorders. Molecular mechanism of action includes mainly inhibition of calcineurin.	(74) (99)
NSAIDs	Ketorolac, Diclofenac Flurbiprofen	NSAIDs inhibit cyclooxygenase enzymes (COX-1 and COX-2) resulting in inhibition of inflammatory mediators such as prostaglandins and leukotrienes.	(4) (2)

Legends:

Fig.1 a) During acute phase of allergic conjunctivitis allergen exposure results in conjunctival MC degranulation and release of a broad spectrum of mediators and cytokines. During late phase, variety of immune cells are recruited to the site of inflammation through chemokine gradient. b) Antigen (Allergen) is phagocytosed, processed and presented by APCs (DCs and macrophages) to T cells. Th2 cells release variety of cytokines by which IgE class switching occurs in Plasma cells. Released IgE molecules bind to FcεR1 on the MC surface. Crosslinking the receptors by allergen results in MC degranulation and a broad spectrum of cytokines and mediators are released. TNFα induces the IL-8 release from conjunctival epithelial cells and fibroblasts which acts through CXCR1 and CXCR2 to make neutrophils infiltrate conjunctiva. Additionally, IL-4 induces Eotaxin-1(CCL11) release from fibroblasts and contribute to eosinophil infiltration by acting through CCR3. DCs upregulate CCR7 in response to CCL21 after antigen uptake and migrate to the ocular lymph nodes to activate T cells.

Fig.2 Epithelial cells induce the release of mediators and chemokines from mast cells by releasing IL-33. Adhesion molecules play a key role in recruiting inflammatory cells to the site of inflammation. VLA-4 expressing inflammatory cells including eosinophils and lymphocytes infiltrate into the conjunctiva via interacting with VCAM-1. Variety of histamine receptors are involved in the development of vasodilation of the ocular surface and facilitate the infiltration of inflammatory cells. IL-33 is capable of inducing cytokine release from T cells.

Fig.3 Fukuda et al reconstituted conjunctival MCs of Kit^{W-sh/W-sh} mice by adoptive transfer of Kit^{+/+} BMCMCs from congenic wild-type C57BL/6J-Kit^{+/+} mice. The cells were tested by flow cytometry and staining to make sure that the majority of cells express CD117 and FcεR1 and contain MC cytoplasmic granules. Passive transfer of RW-specific IgE following by antigen challenge resulted in both early-phase clinical symptoms and late-phase eosinophilic inflammation in Kit^{+/+} mice while both types of responses were decreased in Kit^{W-sh/W-sh} mice. Reconstitution of conjunctival MCs restored early and late-phase responses in conjunctival MC reconstituted Kit^{W-sh/W-sh} mice.

Fig.4 Distribution of histamine receptors on cellular components of conjunctiva. Antihistamines block expression and release of surface molecules and cytokines from conjunctival epithelial cells. The biologic consequences differ based on the activated receptors and expressing cells. For instance, H1 and H2 receptor signaling results in microvascular permeability and production of procollagens. Dual action agents act as H1 receptor antagonists and MC stabilizers. Additionally, immunomodulatory agents act by preventing T cell activation through binding to the intracellular receptor protein cyclophilin-1 interferes with the dephosphorylation and activation of NF-AT. Consequently, the expression of allergic pro-inflammatory cytokines is suppressed.

statement of contribution

Daniel Elieh Ali Komi, Todd Rambasek and Leonard Bielory have been directly involved in the preparation of the manuscript. Daniel Elieh Ali Komi has designed and created the figures. Leonard Bielory has reviewed, revised and added inputs.

statement of conflicts of interest

Daniel Elieh Ali Komi, Todd Rambasek, and Leonard Bielory declare no conflict of interest.







