

REVIEW

Conjunctival cytokines in ocular allergy

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Allergic eye disease is a common clinical problem whose symptoms range from mild to severe, acute to chronic. The milder forms of acute seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC) have symptoms of itch tearing, mucus discharge and redness, which are irritating but not sight threatening. The more severe forms include vernal keratoconjunctivitis (VKC), which affects younger children predominantly boys, usually subsides at puberty, after which it is rarer with an equal sex distribution. Unless adequately treated, it can be sight threatening. Giant papillary conjunctivitis (GPC), is clinically similar, is related to local foreign bodies such as contact lenses, is relieved by their removal, and is not sight threatening. The chronic forms, atopic blepharo-conjunctivitis (ABC) and atopic keratoconjunctivitis (AKC), have a prolonged relapsing sight threatening clinical course, and are often associated with atopic dermatitis and asthma. Sequelae such as herpes simplex infection, often bilateral and devastating, cataracts and glaucoma, are also associated clinical problems.

The mechanism of the whole spectrum of allergic eye has until now been poorly understood, but recent work has advanced our knowledge of the cellular mechanisms involved. The mast cell is central to the whole process, its numbers are increased in all clinical forms [1,2], and it stores, synthesizes and releases the mediators and cytokines responsible for the signs and symptoms of the disease. In all forms of allergic eye disease, the clinical response is due to mast cell activation either directly via antigen-mast cell linkage, or by T-cell activation of mast cells, resulting in mast cell release of inflammatory factors and cytokines. In the milder forms of allergic eye disease, such as SAC and PAC, mast cell numbers alone are increased [3], but in VKC, ABC and AKC, whilst mast cell numbers are increased [1,2,4,5], so are T cells numbers [6]. In the normal subject, conjunctival T cells are naive and levels are low, but in the chronic allergic conditions (AKC, VKC, GPC), there is an increase in CD4 + memory T cells [7]. In

VKC and GPC, 50% of T cells co-express CD45RO and CD45RA, which is rare in AKC and ABC, suggesting that in these conditions which are associated with systemic atopy, T cells are recruited from circulating memory T cells. In VKC and GPC, T cells may be produced locally [7]. Conjunctival mast cells exist in two forms, characterized by their staining pattern to the proteases tryptase and chymase, the MCtc form found in skin, containing both tryptase and chymase, and the MCt form found in mucus membrane surfaces [8] increasing in aero-allergen driven disease, and containing just tryptase [8]. The latter are increased in allergic eye disease. In for instance SAC, mast cell numbers are not only increased, but come to occupy both the deep layers of the conjunctiva, where they are found in non atopic subjects or in out of season SAC, but also the superficial conjunctival layers [epithelium] [2,9,10].

Mast cell activation results from multivalent allergen cross-linking cell surface IgE with high affinity FcεR1 mast cell receptors. Conjunctival mast cell activation leads to the release of histamine and locally synthesized mediators prostaglandin D2, the leukotriene C4, tryptase, chymase, carboxypeptidase-A, cathepsin-G, platelet activating factor (PAF), a powerful eosinophil chemotactic agent and other eosinophil and neutrophil chemoattractants resulting in the recruitment of eosinophils and neutrophils. The signs of allergic conjunctivitis are seen after the topical application of either prostaglandin or the leukotriene LTC4 [11,12]. Allergen challenge in atopic patients leads to increased tear levels of histamine, the mast cell protease tryptase, leukotrienes and eosinophil levels [13,14]. In atopic subjects, allergen challenge leads to an early phase response maximal at 20 min, with raised tear tryptase and histamine levels, [Bacon *et al.* submitted]. At 6 h there is a dose-dependent late phase response, with a second peak of tear histamine and eosinophil cationic protein levels, but not tryptase, suggesting that either mast cells are still degranulated and so refractory to tryptase or that other cells such as basophils, whose conjunctival numbers are increased at this stage, are responsible. As tissue adhesion molecules E-selectin,

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ICAM-1 but not VCAM-1 are increased at 6 h the increased conjunctival levels of granulocytes and eosinophils can be explained. Immunoglobulin (Ig) E is produced in the conjunctiva, under mast cell control, and most allergic eye disease patients have a positive family history of atopy, with raised serum and tear levels of allergen-specific IgE. Mast cells induce B-cell IgE production independently of T cells, suggesting that mast cells may be involved in the auto-regulation of IgE production through the CD40/CD40 ligand-dependant mechanisms.

That the mast cell is responsible for the allergic eye response is shown by the demonstration that the histamine H1 antagonist emedastine [15] potently inhibits the histamine induced secretion of IL-6, IL-8 and GM-CSF [15], and the clinical effectiveness of topical mast cell stabilisers such as sodium cromoglycate, lodoxamide [16], and nedocromil [17], which reduce tear tryptase and inflammatory levels [17]. Topically applied antihistamines such as levocabastine [18], and systemic histamine H1-receptor antagonists such as astemizole, terfenadine and loratidine are also effective in the treatment of SAC and PAC [2].

Mast cell release of histamine and leukotrienes contribute to the inflammatory response by the recruitment of eosinophils and neutrophils. Eosinophil numbers are also increased in the chronic forms of allergic eye disease [19–21]. In AKC and VKC, they are increased not only in the deep layers of the conjunctiva (lamina propria), but migrate into the epithelium [20]. In VKC, there are increased tear levels of eosinophil cationic protein (ECP), eosinophil granule major basic protein and Charcot-Leydon crystal protein. Eosinophil granule-derived proteins have been immunolocalized to the base of vernal ulcers [22]. Neutrophil numbers are also increased in the conjunctiva of atopic subjects after allergen challenge [2, Bacon *et al.*, submitted].

How does the mast cell influence the conjunctival allergic response apart from the release of inflammatory factors? The mast cell has been shown to store, release and synthesize the cytokines IL-4, IL-5, IL-6, IL-8, IL-13 and TNF α [4]. Recent work has shown that the two types of conjunctival mast cell are heterogeneous with respect to cytokine storage [23]. In SAC, IL-4 and IL-13 are predominately localized to MCtc cells, IL-5 and IL-6 to MCt cells [23]. IL-4 promotes T-cell growth, induces the switching of B cells from IgM to IgE production and directs Th2 differentiation essential for the allergic reaction. In the mast cell there are two types of IL-4 present. One form is contained in granules throughout the whole cytoplasm, thought to be the stored form and found in out of season SAC, and the other is found in increased amounts only in the cell membrane in active SAC [24]. IL-4 is also manufactured in the mast cell, for in-situ hybridization techniques using riboprobes to IL-4 mRNA have demonstrated the production of IL-4 within the mast cell [23]. Another role for IL-4 is that of control of adhesion

molecule upregulation. The adhesion molecule VCAM-1 is under the specific influence of IL-4 and is strongly expressed in VKC, but not after allergen challenge in SAC, [Bacon *et al.* submitted]. It has been shown that there is a correlation between the levels of adhesion molecule expression and the different types of inflammatory cell infiltrate in allergic eye disease [24]. All types of adhesion molecules (E-selectin, ICAM-1 and VCAM-1) are expressed in increased amounts only in active allergic eye disease, but not for instance in out of season SAC. There is a positive correlation between ICAM-1 and E-selectin levels and the degree of granulocyte and lymphocyte infiltrate, and VCAM-1 expression with eosinophil levels [24]. Therefore the specific pattern of granulocyte and lymphocyte infiltrate is due to the relative concentration rather the selective recruitment of the different adhesion molecules. Thus in VKC where there are high conjunctival levels of CD4 + cells and eosinophils, VCAM-1 levels are higher than in SAC where CD4 + levels are not raised above normal levels [23,24].

Stem cell factor [SCF] is an essential growth factor for mast cells, and enhances IgE-dependant mast cell mediator release, cytokine generation and release, and is a chemo-attractant for mast cells. It has recently been shown that the mast cell not only stores but manufactures SCF and there is a fourfold increase of SCF in SAC [25], giving the mast cell the potential for its own autoregulation.

TNF α up-regulates endothelial cell adhesion molecules, leading to the recruitment of inflammatory cells. It is a mediator secreting cell priming agent, degranulates mast cells, and is present in mast cells, but conjunctival tissue levels have not been found to be significantly increased in SAC [3]. However using a sensitive radioimmunoassay method, Vesaluoma and colleagues report in this issue, that after topical allergen challenge in atopic subjects, that there is a significant early release of tear TNF α , suggesting that TNF α is an early mediator of the conjunctival allergic response. There is no mention in their work of whether there was also at the same time an increase of tear tryptase levels, which would suggest that the mast cell was the source of the TNF α release. It is produced by other cells such as lymphocytes, neutrophils and eosinophils, all potential sources of TNF α . Future investigations are needed to determine this.

Thus the conjunctival mast cell storage manufacture and release of cytokines results in the signs and symptoms of allergic eye disease, and the therapeutic suppression of these cytokines offers a new approach to its treatment.

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