



Molecular immunology of allergic conjunctivitis

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Purpose of review

Successful treatment of allergic eye disease depends on understanding the pathophysiology of ocular allergy. Thus, in this review, recent experimental and clinical research that has provided significant information about the immunopathology of allergic eye disease will be discussed.

Recent findings

Recently, role of various cells, cytokines and chemokines has been scrutinized in the immunopathogenesis of ocular allergy. In this respect, current research highlights contribution of special molecules and cells in the development and clinical features of immunoglobulin E (IgE) and T-cell-mediated eye allergies.

Summary

Recent findings in molecular immunology of ocular allergy, which comprise complex inflammatory conditions of the conjunctiva, have enabled us to better understand the pathophysiology of these diseases and have aided in the potential development of new therapeutic agents.

Keywords

allergic conjunctivitis, cytokines, eosinophils, mast cells, T lymphocytes

INTRODUCTION

Allergic eye diseases represent a wide spectrum from acute, self-limited, mild form of seasonal/perennial allergic conjunctivitis (SAC/PAC) to chronic, severe, sight-threatening vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) [1,2]. The signs and symptoms of allergic conjunctivitis are influenced by genetics, environmental factors, ocular microbial flora and immune regulation mechanisms, all of which work together in a complex immunological response. Understanding the pathophysiology of ocular allergy is crucial for the successful treatment of allergic eye disease and development of new antiallergic agents.

In this review, recent experimental and clinical research on ocular allergy that has provided us with significant information about the cells, mediators and immunologic pathways having role in the immunopathology of the disease will be discussed.

PATHOPHYSIOLOGY OF ALLERGIC EYE DISEASES

Allergy is an abnormal hypersensitivity response of the immune system to commonly encountered antigens. Allergic eye disease might be caused by an immunoglobulin E (IgE)-mediated hypersensitivity reaction, a T-lymphocyte-mediated hypersensitivity reaction or both [2,3]. SAC/PAC allergic

conjunctivitis is initiated by common allergens binding to IgE on mast cells and triggering the type I hypersensitivity response, whereas in VKC and AKC, T-cell-mediated responses and eosinophil infiltration as well as IgE-mediated hypersensitivity reactions play an important pathogenetic role.

IGE-MEDIATED HYPERSENSITIVITY IN OCULAR ALLERGY

Ocular allergic reactions typically occur in three phases: the sensitization phase, the early phase and the late phase.

The sensitization phase

The sensitization phase occurs upon initial exposure of a genetically predisposed individual to a novel allergen landing on the ocular surface. These

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KEY POINTS

- Seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC) are initiated by common allergens binding to immunoglobulin E (IgE) on mast cells and triggering type I hypersensitivity response, whereas in vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC), Th2-cell-mediated responses and eosinophil infiltration as well as IgE-mediated hypersensitivity reactions play an important pathogenetic role.
- Histamine is the primary mediator involved in ocular allergic response, and of the four known histamine receptors, the subtypes H₁R, H₂R and H₄R have been most strongly linked to ocular allergy.
- Eosinophil infiltration and activation are responsible for the corneal complications in chronic allergic diseases, and eosinophil cationic protein levels show strong correlations with clinical signs of allergy.
- During the active inflammatory phase of allergic eye diseases, multiple Th1-type and Th2-type cytokines were shown to be overexpressed and produced.
- Increased expression of growth factors, TGF- β /Smad signalling pathway and the altered balance between the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of MMP contribute to the excessive deposition of extracellular matrix, the formation of giant papillae and tissue remodelling in VKC.

allergens are phagocytosed by dendritic cells or other antigen-presenting cells (APCs) located in the mucosal epithelium of the conjunctiva and the limbus. Recently, CD11b and CD68-positive macrophages were also shown to act as APCs in experimental allergic conjunctivitis (EAC) [4]. The allergens are processed within the APCs and then presented on the cell surface as a peptide fragment in association with the major histocompatibility complex (MHC) class II molecule. APCs then interact with naïve CD4, or T helper (Th0) cells that express antigen-specific T-cell-receptor (TCR), causing maturation and differentiation of these naïve cells into effector lymphocytes [5]. Increased expression of high-affinity receptor for IgE (Fc ϵ RI) on dendritic cells in the conjunctiva of VKC patients might enhance their ability to capture and internalize antigens for presentation to T lymphocytes and potentiate the allergic response [6]. To achieve full CD4+ T-lymphocyte activation, paired interactions between co-stimulatory molecules B7 on the APCs and CD28 on CD4+ T lymphocytes are necessary. Activated lymphocytes release interleukin 2 (IL-2), which aids in T-lymphocyte differentiation into effector T cells (Th1 or Th2 lymphocytes), as well as memory CD4+ T

lymphocytes. Th2 cells are mainly involved in IgE-mediated allergic response by releasing IL-3, IL-4, IL-5, IL-9, IL-10 and IL-13 that are responsible for IgE production by B cells, mast cell growth, eosinophil accumulation and mucus hyperproduction [7].

When antigen peptide–MHC molecule on B cell and TCR on the CD4+ lymphocyte interacts, adhesion molecules and pairs of co-stimulatory molecules also interact, activating B lymphocytes to proliferate and differentiate into plasma cells, which secrete antigen-specific IgE that binds to its high-affinity receptor Fc ϵ RI located on the surface of mast cells and basophils, completing the process of sensitization.

Within healthy conjunctiva, tight junctions of the epithelium act as a nearly impermeable barrier to the passage of toxins, microorganisms and allergens. Tight-junction proteins include zonula occludens 1 (ZO-1) and calcium-dependent adhesion molecule E-cadherin. House dust mite fecal pellets [8], pollen peptidases [9] and histamine [10²²] are potent disruptors of tight junctions. Consistently with these findings, patients with SAC exhibited a down-regulation of epithelial cell adhesion proteins and cytoskeletal elements [11]. In addition, conjunctival allergen challenge (CAC) has been shown to cause a significant decrease in expression of ZO-1, which was prevented by a topical antihistamine [12].

Protease activated receptor-2 (PAR-2) is abundantly expressed on epithelial surfaces lining the airways, gut and the skin, and alters epithelial permeability by the disruption of epithelial E-cadherin [13,14]. Increased expression of PAR-2 in the conjunctival epithelium of SAC patients exposed to pollen enables antigens to enter the subepithelial space and meet with the mast cells [15²²].

Detection of tear IgE is useful for the diagnosis of allergic conjunctivitis. Recently, a new commercial kit (Immfast Check J1) was introduced for the rapid immunoassay of specific IgE for cedar pollen, cat dander and house dust in serum. In allergic conjunctivitis, using this kit, specific IgE positivity in tear fluid samples for cedar pollen, cat epithelium and *Dermatophagoids pteronyssinus* was found to be 96.9, 23.4 and 53.1%, respectively [16].

Early-phase response

When previously sensitized eye subsequently encounters the same allergen, the allergen attaches to and cross-links IgE–Fc ϵ RI complexes on the surface of mast cells. Subsequent mobilization of intracellular calcium results in degranulation of the mast cells, releasing a variety of preformed inflammatory mediators such as biogenic amines

(histamine), neutral proteases (chymase, tryptase), proteoglycans (heparin, chondroitin sulphate) and acid hydrolase within a few minutes [3]. Early-phase response is characterized by itching, tearing, swelling, oedema and redness, beginning within seconds of allergen contact and lasting for up to 40 min after exposure. Histamine is the primary mediator involved in ocular allergic response [10¹⁷]. Of the four known histamine receptors, the subtypes H₁R, H₂R and H₄R have been most strongly linked to ocular allergy. Histamine signalling through H₁R and H₂R has been shown to increase conjunctival hyperaemia, fibroblast proliferation, cytokine secretion, expression of adhesion molecules, microvascular permeability and production of procollagens [10^{17,18}]. The most recently discovered H₄R modulates a variety of physiological functions, including cytokine and chemokine release, adhesion molecule expression and chemotaxis [19]. VKC conjunctival biopsies display higher levels of H₁R, H₂R and H₄R than does the normal conjunctiva [20²¹]. Histamine receptors are expressed by stromal inflammatory cells and histamine binding to H₄R may selectively recruit mast cells, eosinophils, dendritic cells and lymphocytes into the conjunctiva.

In the second phase, mast cells secrete prostaglandins, thromboxanes, leukotrienes, platelet-activating factor and cytokines which increase conjunctival microvascular permeability, vasodilatation, chemotaxis and activation of neutrophils, eosinophils and additional inflammatory cells, leading to late-phase allergic response [3,21,22].

A candidate treatment approach in allergic diseases is the manipulation of prostanoids and their receptor-signalling pathways, since PGE₂ acting on its receptor subtype EP₃ expressed on the epithelial cells down-regulates the allergic reaction [23]. In EAC model, EP₃-deficient mice demonstrated much more pronounced allergic inflammation with a significantly increased eotaxin expression and eosinophil infiltration in the conjunctiva after allergen challenge compared with wild-type mice [24]. EP₃-selective agonist, ONO-AE-248, was shown to suppress late-phase EAC by decreasing eosinophil infiltration.

Late-phase response

The late-phase response occurs approximately 4–6 h after early-phase response and is characterized by allergic symptoms persisting long after allergen exposure. In CAC model, analysis of conjunctival scraping and tear samples shows an early accumulation of neutrophils, followed by the recruitment of eosinophils within 6–10 h and a later infiltration of lymphocytes [25].

Late-phase response leads to chronic inflammation of the ocular surface and plays a major role in the pathophysiology of the most severe forms of ocular allergic disorders [3,25]. Activated mast cells, conjunctival and corneal epithelial cells and fibroblasts all express and produce cytokines, chemokines and adhesion molecules which initiate the recruitment phase of inflammatory cells into the conjunctiva, leading to the ocular late-phase reaction [21,22,26–29]. In VKC, Th₂ lymphocytes, eosinophils and activated mast cells were shown to be increased in the conjunctival epithelium, subepithelium and tear samples [30,31].

MAST CELLS IN OCULAR ALLERGIC DISEASES

Conjunctival mast cells are essential for early-phase response and eosinophilic inflammation in late-phase response [32,33]. Upon exposure to an allergen, antigen-specific IgE binds to the high-affinity receptor FcεRI on mast cells leading to degranulation of inflammatory mediators. FcεRI activity can be significantly increased via activation of other receptors expressed on mast cells, including CC chemokine receptor 1 (CCR1) [34]. FcεRI–CCR1 costimulation results in up-regulation of expression of several genes and proteins, as opposed to stimulation with antigen or CCL3 alone [35]. Conjunctival mast cells also express eotaxin-1 receptor CCR3 and CCR3 blockade significantly suppresses allergen-mediated hypersensitivity reactions as well as IgE-mediated mast cell degranulation [36].

EOSINOPHILS IN OCULAR ALLERGIC DISEASES

Eosinophil infiltration and activation are responsible for the corneal complications in chronic allergic diseases. When arginine-rich, highly charged toxic proteins released from activated eosinophils bind to basement membrane proteoglycans and hyaluran, they may cause cellular disaggregation and epithelial desquamation, leading to corneal epithelial damage and ulceration in VKC and AKC. Eosinophil cationic protein (ECP) levels showed strong positive linear correlations with clinical signs of AKC, and tear ECP levels were significantly reduced after treatment with tacrolimus [37³⁸].

T CELLS IN OCULAR ALLERGIC DISEASES

T cells play important roles in type I and IV hypersensitivity reactions. T lymphocytes can be classified according to their surface markers as CD4⁺ and CD8⁺ cells and cytokine profiles as Th₁ and Th₂

cells. When T lymphocytes are sensitized by the presentation of antigenic peptide on MHC class II by APCs, they differentiate into effector CD4⁺ T lymphocytes and memory T lymphocytes. In elicitation phase, memory T cells recognize the antigen peptide together with MHC class II molecules on APCs and release Th1-derived cytokines, such as interferon-gamma (IFN- γ), which induces recruitment and activation of macrophage leading to Th1-mediated delayed-type hypersensitivity reaction playing a role in organ-specific autoimmune disorders, immunity to viral infections, intracellular infection, allograft rejection, and contact dermatitis, or Th2-derived cytokines, such as IL-5, which stimulates the chemotaxis and activation of eosinophil to the site of inflammation [38]. In VKC, mainly Th2 cells, whereas in AKC both Th1 and Th2 cells are involved in the immunological response [30,31,39].

Regulatory T cells are also found to be contributors in the pathogenesis of conjunctivitis, since they have an important role in suppressing the allergic response. In EAC, induction of CD4⁺CD25⁺Foxp3⁺ T cells was shown to suppress the development of allergy through stimulation of alpha-galactosylceramide [40].

Recent findings indicate that innate T cells such as natural killer (NK) cells and $\gamma\delta$ T cells also have an important role in allergic diseases [41,42,43[□]]. Apart from their cytotoxic activity, NK cells can produce high amounts of Th1 (IFN- γ) and Th2 (IL4, IL5, and IL13) cytokines, and inhibit or stimulate the allergic response by specific patterns of cytokine release. In VKC, NK cells decrease in the blood and increase in the conjunctiva, which might indicate a potential role in the regulation of allergic reactions [41]. $\gamma\delta$ T cells were shown to be necessary for secretion of IL-4, IL-5, and IL-13 and Th2-mediated inflammation, and for full expression of early and late-phase allergic conjunctivitis. In EAC, $\gamma\delta$ T ^{-/-} mice had decreased clinical signs of allergic conjunctivitis and reduced eosinophilic infiltration into the conjunctiva compared with wild-type mice [43[□]].

CYTOKINES IN OCULAR ALLERGIC DISEASES

Cytokines contribute to disease pathology through the recruitment and activation of leukocytes and to pro-fibrotic/remodelling events in chronic allergic diseases [44]. Chemokines are cytokines that recruit cells of the immune system to inflammation area. During the active inflammatory phase of allergic eye diseases, multiple Th1-type and Th2-type cytokines and chemokines including IL-4, IL-5, IL-8, IL-10, IFN- γ , tumour necrosis factor-alpha (TNF-

α), monocyte chemotactic protein-1 (MCP-1), RANTES, eotaxins and thymus, and activation regulated chemokine (TARC or CCL17) were shown to be overexpressed and produced [28,29,45–49]. IFN- γ was significantly correlated with corneal involvement [45].

IL-33, a recently discovered cytokine, induces IgE production and eosinophil infiltration to the target tissues, by the signalling cascade through membrane-bound IL-33R. In AKC, IL-33 was found to be expressed by vascular endothelial cells and conjunctival epithelium of the giant papillae, IL-33R was expressed by mast cells, and IL-1 β stimulation up-regulated IL-33 mRNA expression in conjunctival fibroblasts [50].

ROLE OF EPITHELIAL CELLS AND FIBROBLASTS IN OCULAR ALLERGIC DISEASES

Conjunctival and corneal epithelial cells and fibroblasts may contribute to mounting the allergic inflammation by expressing and producing cytokines, chemokines, adhesion molecules, and factors that maintain local inflammation and lead to tissue remodelling [26–29,51,52]. Recently, thymic stromal lymphopoietin, an IL-7 like cytokine, was found to be highly expressed in the conjunctival epithelium of patients with VKC and AKC and shown to activate dendritic cells and mast cells in synergy with other cytokines [53].

Fibroblasts may play a key role in the induction and amplification of ocular allergic inflammation and the consequent development of corneal lesions in chronic eye allergies. Pro-inflammatory and Th2 cytokines including TNF- α , IL-4, and IL-13 stimulate the synthesis of chemokines (eotaxin-1, TARC, RANTES, IL-8) as well as of adhesion molecules [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] in corneal or conjunctival fibroblasts, all of which together promote the activation and infiltration of eosinophils and Th2 lymphocytes [28,29].

STRUCTURAL CHANGES AND MATRIX METALLOPROTEINASES IN OCULAR ALLERGIC DISEASES

An increased expression of growth factors, including transforming growth factor-beta (TGF- β), vascular endothelial growth factor, fibroblast growth factor, and platelet-derived growth factor, has been shown previously in VKC conjunctival tissues and tears [46,54,55[□]]. Sma and Mad-related proteins (Smad) modulate extracellular matrix gene expression during wound healing, inflammation, and tissue

remodelling. TGF- β /Smad signalling pathway is over-expressed in VKC tissues and modulated in conjunctival fibroblasts by histamine, IL-4, TGF- β 1, and TNF- α [55].

The altered balance between the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of MMP (TIMP) contribute to the excessive deposition of extracellular matrix and the formation of giant papillae [46,56]. MMP-1, MMP-2, MMP-3, MMP-9, and MMP-10 were shown to be present in tear samples of VKC patients, whereas only TIMP-1 and TIMP-2 were found in normal individuals [46]. MMP-9 activity correlated significantly with corneal involvement and giant papillae formation [56]. Immunostaining for MMP-9 has been found to be increased in conjunctival eosinophils and in the corneal stroma at the base of ulcers in patients with VKC.

TOLL-LIKE RECEPTORS AND OCULAR ALLERGY

Toll-like receptors (TLRs) are expressed at the ocular surface where they trigger an immediate innate response specific to pathogenic strains and activate adaptive immunity [57–59]. Interestingly, TLR4 was shown to be up-regulated, TLR9 was down-regulated, and TLR2 was slightly reduced in the conjunctiva of VKC patients, suggesting that TLRs play a role in the pathogenesis of VKC [60,61]. It has also been shown that TLR2 expression was increased in AKC conjunctiva [62]. In EAC, administration of endotoxin lipopolysaccharide (LPS) was shown to suppress IgE-mediated and eosinophil-dependent conjunctival inflammation via the TLR4-dependent pathway [63]. Mice sensitized with ovalbumin (OVA) and LPS had less IL-4, IL-5, and eotaxin secretion than mice sensitized with OVA only.

NEUROGENIC PATHWAY IN OCULAR ALLERGIC DISEASES

There is growing evidence showing a role of peripheral nervous system in allergic inflammatory cascade, through the release of neuromediators, including substance P, neuropeptide Y, vasoactive intestinal peptide, calcitonine gene-related peptide, and nerve growth factor (NGF) [64]. NGF released by inflammatory cells intervenes in the neurogenic inflammatory process of allergic phenomena by modulating Th and B-lymphocyte proliferation and stimulation, and increasing the functional activity of mast cells and eosinophils [65]. Neuro-mediators are normally present in tears of healthy individuals. Tear levels of neuromediators were shown to increase significantly after CAC in allergic

patients [66] and VKC [67]. Also an increased and altered expression of muscarinic receptors, adrenergic receptors, and neurotransmitters in the conjunctiva has been described in patients with VKC [67]. NGF was shown to induce TLR4/TLR9 overexpression in conjunctival epithelial cells in VKC [61].

CONCLUSION

Recent findings in molecular immunobiology of ocular allergy, which comprise complex inflammatory conditions of the conjunctiva, have enabled us to better understand the pathophysiology of these diseases and have aided in the potential development of new therapeutic agents.

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None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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