Allergic Conjunctivitis: Update on Its Pathophysiology and Perspectives for Future Treatment

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Introduction

Allergic conjunctivitis is one of the most common syndromes that a general ophthalmologist is presented with, with a prevalence ranging from 5% to 22% in the general population [1, 2].

Ocular allergic diseases share some common eye symptoms and signs such as redness, itching, tearing and discharge. However, symptoms and signs represent the final clinical outcome of different pathophysiological mechanisms that are peculiar to different phenotypes of allergic eye disease [3, 4].

Four Forms of Allergic Conjunctivitis: A Clinical Simplification

Allergic eye disease, in fact, includes a spectrum of different clinical entities with variable presentation. The milder and the most common forms are seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC). Generally, SAC and PAC patients complain of symptoms such as itching, tearing, mucus discharge and redness, but these two forms are not sight threatening. On the contrary, more severe forms of ocular allergies, such as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC), can involve the cornea and may be sight threatening if not promptly diagnosed and adequately treated.

If three are the "classic" phenotypes of allergic conjunctivitis, two are the main pathological pathways usually considered as the basis of these forms: conjunctival

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R. Pawankar et al. (eds.), *Allergy Frontiers: Clinical Manifestations*,
DOI: 10.1007/978-4-431-88317-3_2, © Springer 2009

mast cell activation and eosinophil recruitment into the ocular surface. Indeed, ophthalmologists know that such a plain classification and simplified pathophysiological description do not adequately account for the complexity of diagnostics and treatment in clinical settings [5]. Moreover, the biological mechanisms at the base of ocular allergy seem to be distinct from those involved in allergic diseases that affect other organs in the body. This complexity is attributable to the unique immunological characteristics of the anterior segment of the eye, and to the specific mechanisms by which the structural cells (i.e., epithelial cells and stromal fibroblasts) interact with the inflammatory cells infiltrating the conjunctiva.

Seasonal and Perennial Allergic Conjunctivitis

Seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC) account for 95% of the allergic eye diseases in the practice. These forms involve both eyes and occur seasonally (spring, fall) or perennially (year round), respectively, and do not induce severe ocular surface damage [6].

SAC (Hay fever conjunctivitis) is usually an acute or subacute disease that is characterized by peaks of self-limiting signs and symptoms (i.e., red eye, tearing, itching and mucous discharge), and is mostly due to pollens (e.g., grass, trees, ragweed) that appear during specific seasons.

PAC is less common than SAC and is related to animal dander, dust mites or other allergens that are present in the environment year-round. The symptoms are present all year with seasonal exacerbation depending on the individual sensitization. The hallmarks of this form are itching, redness and puffy eyes. Patients may also complain of tearing, mucous discharge, burning and swelling. However, no symptom or sign is specific to SAC and PAC [7, 8] and seasonal allergens may cause PAC as perennial allergens may be responsible for seasonal forms. Accordingly, the classification in the intermittent and persistent forms may result more appropriately than that in SAC or PAC.

Immunopathogenesis of SAC and PAC

Allergic inflammation of the conjunctiva is a type I hypersensitivity, an immediate reaction associated with IgE-mediated cell activation.

The first step of the process is sensitization: small (picograms) quantities of environmental allergens such as pollens, dust mite fecal particles, animal dander, and other proteins reach the conjunctival mucosa. Here, these particles are processed by Langerhans, dendritic or other antigen-presenting cells (APCs). Proteolitically cleaved antigens subsequently bind to the antigen-recognition site of the major histocompatibility complex (MHC) class II molecules. Carried by APCs, the antigens are then presented to native Th0 lymphocytes that express antigen-specific recep-

tors and recognize the antigenic peptides. This process probably occurs at the local draining lymph nodes. Multiple contacts and cytokine exchanges between APC and T cells are necessary to induce a Th2-type reaction. The cytokines released by the type-2 helper T-lymphocytes (interleukin-3, IL-4, IL-5, IL-6, IL-13 and granulocyte-macrophage colony stimulate factor – GM-CSF) stimulate the production of IgE by B cells.

The second step of the pathophysiology of conjunctival allergy is the triggering, in the sensitized host, of the mast cells residing in the conjunctival mucosa and bearing specific IgE antibodies on the cell surface with the help of high affinity receptors. Exposure to environmental allergens in sensitized individuals causes the cross-linking of IgE at the mast cell membrane level, with subsequent cell degranulation and release of histamine, tryptase, prostaglandins and leukotrienes. These mediators trigger clinical manifestations of the acute phase of the disease (early phase). Mast cell degranulation, however, also induces activation of vascular endothelial cells, and thus expression of chemokine and adhesion molecules, such as: 'Regulated-upon-Activation Normal T-cell Expressed and Secreted' (RANTES), monocytes chemotactic protein-1 (MCP-1), intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM) and p-Selectin and chemotactic factors (IL-8, eotaxin). These factors initiate the recruitment phase of activated inflammatory cells in the conjunctiva. The late-phase reaction to allergen stimulation occurs hours after allergen exposure and is characterized by the recurrence or prolongation of symptoms due to the infiltration of eosinophils, neutrophils and T lymphocytes into the mucosa (Fig. 1). The late-phase reaction plays a major role in the pathophysiology of the most severe forms of ocular allergic disorders [9–12].

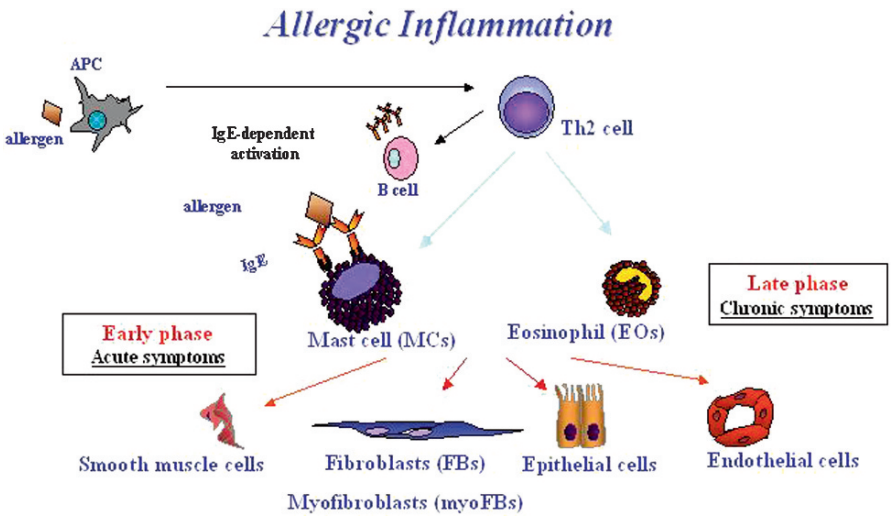

Fig. 1 The early and late phase of ocular allergic reactions

Vernal and Atopic Keratoconjunctivitis

Vernal keratoconjunctivitis (VKC) is a severe allergic disease of childhood with a higher prevalence in male subjects living in warm climates [13]. It is characterized by the presence of corneal epithelial and stromal lesions as well as of conjunctival proliferative changes (i.e., the giant papillae of the upper tarsal conjunctiva) and limbal abnormalities. Patients with VKC are usually, but not necessarily, sensitized to the most common allergens, such as grass, Parietaria and Dermatophygoides. It has been suggested that VKC represents a phenotypic model of overexpression of the cytokine gene cluster on chromosome 5q [14]. This chromosomal area includes genes that regulate the expression of IL-3, IL-4, IL-5 and GM-CSF. The up-regulation of these factors is critical in modulating Th2 prevalence, IgE production, as well as mast cell and eosinophil function.

Two clinical variants of VKC are usually described: the limbal and the tarsal forms. The hallmarks of these two pathologic entities are the giant papillae on the upper tarsal conjunctiva and the gelatinous limbal infiltrates, respectively [15]. Both forms of VKC are characterized by intense itching, tearing, mucous discharge and severe photophobia, that often force children to live virtually in the dark. The intense foreign body sensation is due to the conjunctival surface irregularity and the copious mucous secretion. The onset of ocular pain is indicative of cornea involvement, which can be present in the form of superficial punctate keratitis, epithelial erosions or ulcers and plaques. The different prevalence between genders, and its resolution with puberty, are features that have suggested a role of hormonal factors in the development of VKC [16].

Atopic keratoconjunctivitis (AKC) occurs more frequently in men aged 30–50 years [17]. A family history of allergies, asthma, urticaria or hay fever is often present. Typically, patients have atopic dermatitis or eczema from childhood, but develop ocular symptoms later in life. These symptoms are represented by an intense bilateral itching of the eyes and of the skin of the lids and periorbital areas. Tearing, burning, photophobia, blurred vision and a stringy, rope-like mucus discharge are also observed.

Tylosis and swollen eyelids with a scaly indurate appearance and meibomiam gland dysfunction with associated dry eye are the signs of atopic blepharitis. The conjunctiva can be hyperemic and edematous, and tarsal conjunctival papillae are commonly seen [18].

Immunopathogenesis of VKC and AKC

Vernal keratoconjunctivitis is traditionally thought to be an allergic disorder. The role of an IgE-mediated hypersensitivity in VKC is one of the essential pathogenic steps [19], supported by seasonal incidence, association with other allergic manifestations, increased number of conjunctival mast cells and eosinophils, high levels

of total and specific IgE and others mediators in serum and tears [20] and the therapeutic response to mast cell stabilizers in mild cases of VKC [21, 22]. However, the fact that specific sensitization is not found in many patients, suggests that additional mechanisms, apart from a typical type I hypersensivity mechanism, contribute to the pathogenesis of conjunctival inflammation in VKC patients.

Role of Eosinophils in Chronic Ocular Allergy

Selective infiltration of eosinophils is one of the characteristics of all the forms of allergic conjunctival diseases [23]. In normal individuals, eosinophils are not found in the conjunctival epithelium, although a small number of these cells is present in the substantia propria of the conjunctiva [24]. On the contrary, eosinophils are markedly increased in the substantia propria and infiltrate the conjunctival epithelium in VKC [25]. Eosinophils in VKC are activated, as shown by the expression of eosinophil cationic [26, 27]. Activated eosinophils release cytotoxic proteins such as MBP-1, eosinophil peroxidase, eosinophil-derived neurotoxin and eosinophil cationic protein, and the concentration of these proteins is increased in the tear fluid of these individuals [28–31]. Proteolytic enzymes, cytotoxic proteins and oxygen radicals released by neutrophils contribute to the exacerbation of corneal damage [32, 33]. Corneal fibroblasts are stimulated by neutrophils and participate in collagen degradation, which leads to the subsequent corneal ulceration [33, 34]. This evidence suggests that the interactions between the immune cells and the corneal resident cells play a major role in the pathogenesis of corneal involvement in VKC. The infiltration and degranulation of eosinophils at the limbus are also responsible for the disruption of the corneal epithelium [32]. Moreover, the corneal plaque (also called shield ulcer), which develops in patients with severe VKC [35] is composed of debris derived from eosinophils and epithelial cells [36, 37]. Extravasation of immune cells is regulated by the chemokines expressed by vessels and other structural cells, such as fibroblasts and smooth muscle cells [38]. Although many bioactive substances, including complement C5a [39], leukotriene B4 [40, 41] and platelet activating factor (PAF) are able to induce local infiltration of eosinophils, other factors, in particular chemokines, may also activate different types of immune cells. Most chemokines belong to the CC or CXC sub-families [42–44], with only a few C and CX3C chemokines having been identified to date. In general, CC chemokines mostly induce the infiltration of eosinophils or lymphocytes, whereas, CXC chemokines mostly induce the infiltration of neutrophils or monocyte-macrophages [45]. Eosinophils express the CC receptors CCR1 and CCR3 on their surface. CCR1 docks several chemokines, that is, RANTES, macrophage inflammatory protein-1a (MIP-1a), monocyte chemoattractant protein (MCP-2) and MCP-3. CCR3 binds to RANTES, MCP-2, MCP-3 and eotaxin. For example, the chemokine RANTES induces the local infiltration of eosinophils through interaction with CCR1 and CCR3 [46, 47]. Indeed, the signalling mediated by CCR3 is more effective than the one mediated by CCR1 [42, 48, 49]. CCR3

is expressed by mast cells [50, 51] basophils [52, 53], Th2 lymphocytes [54] and eosinophils [51, 55, 56], but is not present in neutrophils [57]. One of the most potent eosinophil chemoattractant, the eotaxin, binds specially to CCR3 [58–61]. This small protein is synthesized by a number of different cell types, and is stimulated by interleukin-4 and interleukin-13, which are produced by T-helper type-2 lymphocytes [62].

Eotaxin and Corneal Involvement in Ocular Allergy

It is believed that VKC, like other allergic diseases, is a Th2-dominant condition [63, 64]. The cytokines produced by T helper type-2 lymphocytes are IL-4, IL-5, IL-10 and IL-13, and play a pivotal role in the pathogenesis of corneal damage in VKC.

It has been demonstrated that the stimulation of corneal fibroblasts by the Th2 cytokines IL-4 and IL-13 results in a marked release of eotaxin. In fact, corneal fibroblasts are the most significant source of this strong eosinophil chemoattractant, among the structural cells of the ocular surface [65]. The eosinophils present in the conjunctiva in VKC may release a substance that induces a breakdown of the barrier function of the corneal epithelium [66]. Corneal fibroblasts may then be exposed to different factors present in the tears of VKC patients, including TNF-a [67, 68], IL-4 [69] and IL-13. Moreover, the barrier function of the corneal epithelium is diminished in individuals with atopic dermatitis [70], which is often associated with VKC. Activated corneal fibroblasts and the subsequent release of eotaxin may induce a subsequent marked infiltration of eosinophils in the cornea. Breakdown of the barrier function of the corneal epithelium is thus probably a key event in the exacerbation of ocular allergic inflammation.

The Interactions Between Conjunctiva and Cornea in Ocular Allergy

Allergic inflammation begins in the conjunctiva, in which immune cells, such as, lymphocytes, mast cells and eosinophils are increased in number. These cells release Th2 cytokines (TNF-a, IL-4 and IL-13) that also stimulate the conjunctival fibroblasts to produce eotaxin. The chemotactic effect of this chemokine enhances eosinophil infiltration into the conjunctiva. The corneal epithelium is damaged by eosinophil-derived cytotoxic proteins, resulting in the impairment of its barrier function and consequent exposure of the corneal fibroblasts to the bioactive substances present in the tears. The corneal fibroblasts release eotaxin into the tear fluid in response to stimulation with TNF-a, IL-4 and IL-13. Allergic inflammation is then exacerbated as a result of eosinophils and other immune cells infiltrating the cornea and the conjunctiva, thus completing the cycle. Loss of the barrier function of the corneal epithelium and exposure of corneal fibroblasts to the bioactive substances in the tear film is likely to exacerbate the ocular allergy in additional

ways, including induction of the release of other chemokines such as thymus- and activation-regulated chemokine (TARC or CCL17) and matrix metalloproteinase-2 (MMP-2) by corneal fibroblasts.

The giant papillae in VKC manifest a dense infiltration of eosinophils immediately beneath the denuded conjunctival epithelium, corresponding to the location of the Trantas' dots. Eosinophils thus probably migrate from the conjunctiva into the tear fluid and ocular discharge. Such migration may indicate that the concentration of eosinophil chemoattractant in tear fluid is greater than that in the conjunctiva. The release of RANTES and IL-8 by corneal fibroblasts is markedly stimulated by pro-inflammatory cytokines, such as TNF-a [46, 71]. The concentration of these cytokines is increased in the tears of individuals with a variety of ocular inflammatory conditions. These cytokines might contribute to ocular inflammatory reactions regardless of the causative factor [72, 73]. Increased concentrations of several pro-inflammatory cytokines and IL-4 (and possibly IL-13), on the other hand, may be a specific finding of VKC among ocular allergic diseases [66].

A Closer Look at the Cytokine Cascade in Ocular Allergy

Local infiltration of specific types of leukocytes is controlled by the interaction of these cells with adhesion molecules following the chemoattractive effects of chemokines. Adhesion molecules expressed on the surface of vascular endothelial cells facilitate the transmigration of immune cells. Among these adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) play a prominent role [38]. ICAM-1 contributes to the local infiltration of the immune cells – including neutrophils, eosinophils and lymphocytes – during the inflammatory responses. VCAM-1 interacts with very late antigen-4 (VLA-4), which is expressed on the surface of eosinophils and lymphocytes. Inhibition of the VCAM-1–VLA-4 interaction thus suppresses eosinophil infiltration in allergic animals [74, 75]. ICAM-1 expression on human corneal epithelial cells is increased by stimulation of the cells with TNF-a in a concentration-dependent manner [76]. Furthermore, exposure of corneal fibroblasts to IL-4 or IL-13 in the presence of TNF-a induces a synergistic increase in the expression of VCAM-1 [77]. Corneal fibroblasts, but not corneal epithelial cells, up-regulate VCAM-1 expression in a synergistic manner in response to stimulation with TNF-a and either IL-4 or IL-13 [66]. These observations support the importance of the synergistic effects of these cytokines on corneal fibroblasts in the pathogenesis of VKC.

In addition to promoting eosinophil infiltration into the tissue, VCAM-1 induces the activation of these cells, increasing their survival [8, 78] superoxide generation [79], and leukotriene C4 secretion [80]. These effects of VCAM-1 may also contribute to the pathogenesis of allergic eye diseases.

The Th2 cytokines IL-4 and IL-13 are central mediators of allergic diseases [81, 82]. IL-4 and IL-13 regulate biological responses by binding to specific IL-4 receptors (IL-4Rs) expressed by a wide range of cell types, including T and B lymphocytes, monocytes, granulocytes, endothelial cells, epithelial cells and fibroblasts

[83–86]. The combination of TNF-a with either IL-4 or IL-13 induces the release of eotaxin and expression of VCAM-1 by corneal fibroblasts [66]. The IL-4Ra chain is the functional subunit of IL-4R complexes that mediates the activation of STAT6 [85, 87–90]. STAT (signal transducer and activator of transcription) proteins are intracellular signalling molecules that are activated on exposure of cells to various cytokines, growth factors or hormones. STAT6, one of the seven known mammalian members of the STAT family, is phosphorylated and activated in response to IL-4 or IL-13. Phosphorylated STAT6 molecules form dimers that translocate to the nucleus, where they activate transcription of target genes. The promoter of the eotaxin gene contains consensus-binding sites for STAT6 [91], suggesting that the effects of IL-4 and IL-13 on eotaxin expression in corneal fibroblasts might be mediated at the transcriptional level by the IL-4R-STAT6 signalling pathway. STAT6 knockout mice exhibit defects in various IL-4-mediated functions, such as induction of the expression of CD23 and major histocompatibility complex class II genes, Ig class switching to IgE, proliferation of B and T cells, and Th2 cell development, demonstrating the importance of STAT6 in IL-4 signalling [92, 93].

The Th1 and Th2 Paradigm in Ocular Allergy

Type-2 T helper lymphocytes (Th2) are thought to play a key role in the development of allergic disorders by producing regulatory and inflammatory cytokines such as, interleukin-4 (IL-4), IL-5 and IL-13. These cytokines have been found in tears and tissues of patients affected by VKC and AKC [94–98]. Although there is no doubt in the role played by Th2 cells in ocular allergy, a Th1 response has also been demonstrated [99]. In fact, it is not clear if the expression of the IFNγ – a Th1-type, proinflammatory cytokine – in chronic ocular allergic disorders is an attempt to down-regulate the Th2 response or a separate inflammatory pathway.

IFNγ, IL-4 and IL-13 may be produced and expressed together in ocular inflammatory allergic responses [99, 100]. Both VKC and AKC tears contain higher levels of IL-4 and IL-13 than normal tears [32]. IFNγ, increased in the tears of patients with corneal damage, significantly correlates with the corneal score, suggesting that the overproduction of this pro-inflammatory cytokine might be related to a worsening of the allergic inflammation. Conversely, in peripheral blood mononuclear cells of allergic patients, the frequency of the IL-4-producing T-cell is increased compared with that of healthy subjects, indicating that Th1 cells are only locally activated [101]. It has been proposed that Th1 cells protect against allergic disease by dampening the activity of Th2 lymphocytes. In fact, Th1 cells inhibit the proliferation and development of Th2 cells and IFNγ inhibits IgE synthesis [102–105]. IFNγ-secreting cells may be important in perpetuating chronic inflammation, since this cytokine up-regulates the expression and production of adhesion molecules, chemokines and co-stimulatory molecules by conjunctival [104] and corneal [105] epithelial cells [106].

IFNγ has been reported to up-regulate VCAM-1 expression on endothelial cells [107]. Ocular surface irritation due to external stimuli results in an immediate

response from ocular surface conjunctival epithelial and endothelial cells, which includes the secretion of pro-inflammatory mediators at the site of injury. These pro-inflammatory mediators stimulate endothelial cell expression of adhesion molecules (e.g., VCAM-1), which are necessary for migration of immune cells to the ocular surface. In other words, IFNγ acts as a gatekeeper by inducing the expression of VCAM-1 to facilitate cellular extravasation into the injured tissue and enhancing the recruitment of immune cells [100]. In fact, the absence of IFNγ significantly reduces eosinophil migration into the conjunctiva. In conclusion, IFNγ may play an important role in ophthalmic Th2-type inflammation because it may control the capability of immune cells to gain entry into the extravascular tissue in both Th1 and Th2 response.

Both type 1 and type 2 cytokines are present in the tears during the active phase of severe SAC, VKC and AKC [108]. Even in SAC there is an increase of IL-1, IL-2, IL-4, IL-5, IL-6, IL-12, IL-13, IFNγ and MCP-1, suggesting that mast cells are not the only immune cells involved. There is a positive correlation between the percentages of tear-containing lymphocytes and IL-12 and IL-13 levels, whereas, no association is found with eosinophils [109]. This is surprising and suggests a possible general over-estimation of the role of eosinophils in ocular allergy. On the other hand, the conjunctival fibroblasts are receiving increasing attention for their potential contribution to the pathogenesis of allergic eye diseases. In fact, conjunctival fibroblasts constitutively produce IL-6, IL-8, MCP-1 and RANTES [108] and release eotaxin when stimulated with IL-4. The expression of CXC chemokines (IP-10, Mig) by conjunctival fibroblasts in response to pro-inflammatory cytokines, further supports a major role of these cells in the recruitment of T cells during chronic allergic eye disease [108].

Although SAC, VKC and AKC tears contain different levels of cytokines, these forms of conjunctivitis do not show a disease-specific Th1 or Th2 profile. Results of studies on tears reveal that allergic diseases differ predominantly in the quantity, rather than the quality, of cytokines present in tears as a result of complex interactions or mutual regulation.

Tissue Remodeling in Chronic Ocular Allergy

Fibroblasts and epithelial cells are not a simple target of ocular allergy, but play a pivotal role in the initiation and modulation of inflammation in the tissues by attracting and activating specific sets of immune cells [65, 110, 111]. In particular, conjunctival and corneal fibroblasts participate in the pathogenesis of ocular inflammation in severe forms of allergic keratoconjunctivitis, such as VKC [112], and they contribute to the formation of corneal ulcers inducing the degradation of collagen [33]. For this reason, nowadays, ocular fibroblasts represent a potential target for new therapeutic approaches to severe ocular allergy.

From a molecular point of view, corneal fibroblasts, stimulated by the combination of TNF-a and either IL-4 or IL-13, release TARC (CCL17) and the

macrophage-derived chemokine (MDC or CCL22). These two CC chemokines are potent and selective chemoattractants for Th2 lymphocytes [113, 114], that express the corresponding receptor CCR4 on their surface [115, 116]. The local release of TARC and MDC contributes to the maintenance of allergic inflammation through the promotion of Th2 cell infiltration. This process is then amplified by the incoming inflammatory cells that cooperate with different mechanisms, to the Th2 response started by the tissue resident cells, that is, fibroblasts and epithelial cells.

The structure and functions of the corneal epithelium are regulated by the underlying basement membrane. Changes in the components of the basement membrane cause epithelial defects and corneal ulcers [117]. Type IV collagen and laminin are predominant components of the basement membrane of the corneal epithelium [118]. These two proteins are specifically degraded by MMP-2 and MMP-9. MMPs are released from various types of cells as latent proenzymes, which undergo proteolytic cleavage to generate the active form of each enzyme. The activities of MMPs are also down-regulated by tissue inhibitors of metalloproteinases (TIMPs).

The tears normally contain the pro forms of MMP-2 and MMP-9, but not the active forms. The presence of activated MMP-2 and MMP-9 in the tear fluid of VKC patients suggests that these proteins may induce degradation of the basement membrane, contributing to the formation of corneal ulcers. Corneal epithelial cells and fibroblasts are the probable source of lachrymal MMP-2 and MMP-9, because both these cell types constitutively express these MMPs and release them in response to stimulation by pro-inflammatory cytokines such as TNF-a and IL-1 [119]. The increased expression of MMP-2 and MMP-9 by resident cells of the cornea also prolongs reepithelialization of the cornea after injury [117].

Giant tarsal papillae and limbal Trantas dots are characteristic proliferative changes of the conjunctiva in VKC [15]. These lesions are mainly constituted by collagen types I and III and fibronectin, and are infiltrated by eosinophils, mast cells, Th2 lymphocytes and fibroblasts [120]. Conjunctival fibroblasts, balancing the synthesis and degradation of the extracellular matrix (ECM), control the metabolism of ECM proteins and proteoglycans and maintain the normal tissue structure. In vitro, Th2 cytokines IL-4 or IL-13 induce the proliferation of cultured conjunctival fibroblasts, increase the deposition of fibronectin and collagen types I and III by inhibiting the production of MMP-1 and stimulate their production of TIMP-1 [68]. An increased synthesis of collagen by the fibroblasts may lead to conjunctival hypertrophy and fibrosis in VKC.

Collagen and fibronectin not only provide structural support to cells, but also function as signalling molecules, playing key roles in allergic inflammation by regulating the activation of infiltrating immune cells. For instance, the interaction of eosinophils with the ECM facilitates their survival and activation, while the attachment of monocytes or macrophages to ECM stimulates their expression of IL-6 and TNF-a [121]. The ECM also serves as a reservoir for cytokines and growth factors: Transforming growth factor beta (TGF-b), basic fibroblast growth factor (bFGF) and the granulocyte-macrophage colony-stimulating factor (GMC-SF) bind with theECM components, and the ECM stabilizes or increases the local concentration of these growth factors.

IL-4 or IL-13 stimulate conjunctival fibroblasts to secrete ECM proteins such as collagen and fibronectin during allergy. In this way, fibroblasts increase the retention of inflammatory cytokines by the conjunctival stroma and further activate inflammatory cells. All these mechanisms induce subepithelial fibrosis and proliferation of capillaries that provide vascular support to the giant papillae in VKC. Ialin degeneration of conjunctival stroma and mucus metaplasia are also signs of chronic, severe ocular allergy [15].

The Emerging Role of the Innate Immune System, Toll-Like Receptors and Allergic Conjunctivitis

The innate immune system allows a fast and proper immune response to limit or completely destroy the invading pathogens [122]. Toll-like receptors (TLRs) play a crucial role by recognizing proteins or DNA/RNA sequences belonging to bacteria, protozoa, helminths, fungi and viruses [122]. Specific TLR activation, results in the production of pro-inflammatory mediators and cytokines, driving an antimicrobial host response [123].

TLRs are transmembrane type I glycoproteins characterized by an extracellular leucine-rich domain and a cytoplasm tail, homologous to the signal domain of the IL-1 receptor, which predominantly mediate the activation of mitogen-activated protein kinase and nuclear factor kappa B/activator protein-1 pathways and lead to cell activation and differentiation [123]. Eleven human TLRs have been characterized so far and classified according to their specific natural agonists: in general, TLR-2 and TLR-4 recognize bacterial products, whereas, TLR-3, TLR-7, TLR-8 and TLR-9 are principally designed to join nucleic acids [123]. A hallmark of the cell's response to the activation of innate immune systems is the release of TNF-[alpha], IL-1[beta] and IFN-[gamma] cytokines [123].

Innate/adaptive cross-talk has recently been demonstrated to be driven by TLR expression on APCs and structural cells of the ocular surface [124]. Ocular surface inflammation results from complex interactions between innate and adaptive responses. TLR expression has been found in the corneal and conjunctival epithelia, and this expression seems to be affected during bacterial/viral infections as well as in allergic conditions [124]. The presence of TLRs in the ocular epithelium may be relevant for defence mechanisms towards microbial agents in contact with the ocular surface. It has been hypothesized that commensal flora may be critical for the maintenance of epithelial mucosal homeostasis, by playing a paradoxically protective role after epithelial injury [124]. Moreover, changes in the commensal flora might influence the immune response in disease states. Certain micro-organisms may thus actually be important for protection against allergy [125].

Corneal epithelial cells express TLR-4 and co-stimulatory molecules (CD14, MD2), and stimulation by TLR-4 agonists results in pro-inflammatory cytokine and chemokine secretion [126]. Interestingly, the corneal epithelium does not normally respond to commensal flora, although this is commonly present in the tear film, as observed by the fact that patients suffering from bacterial conjunctivitis do not

display corneal inflammation. The corneal epithelium appears to posses a unique way (intracellular localization) to modulate the functional activity of the highly expressed TLR-2 and TLR-4, and therefore to control unnecessary inflammation. In fact, corneal epithelial cells do not express TLR-2 and TLR-4 at the cell surface, failing to elicit immune response to ligands [127]. In a recent study, the role played by TLR-4/CD14/MD-2-expressingfibroblasts (activated keratocytes) in lipopolysaccharide-induced inflammation associated with bacterial corneal ulceration, has been demonstrated [128]. In agreement with its role as a first line of defence, the healthy conjunctival epithelium expresses high levels of TLR-9, compared with the average expression of TLR-2 and TLR-4, whereas, the expression by the underside stroma is at similar levels [129]. This expression is modified in patients with VKC, as demonstrated by our group [124, 129]. Real-time evaluation of VKC conjunctiva showed a significant up-regulation of TLR-4 and down-regulation of TLR-9, with a slight reduction in TLR-2, compared with healthy conjunctiva. Confocal analysis showed that in VKC, stromal TLR-4 expression was mainly caused by fibroblasts, infiltrating eosinophils and mast cells. High levels of TLR-4 expression in VKC tissues is substantiated by previous reports correlating TLR-4 expression to the allergic phenotype.

The hypothesis that early life-specific activation of TLRs may contribute to a more balanced T helper type 1/2 response, avoiding over-activation of the T helper type 2 pathway, has been proposed [130, 131]. In line with this hypothesis, further studies on the role of commensal flora in influencing innate immunity in the eye, mainly during the early stages, may lead to a re-evaluation of the mechanisms that activate the adaptive immune responses after microbial ocular infections (Fig. 2). Finally, pharmacological activation and regulation of TLRs may also offer new therapeutic alternatives for the modulation of allergic and immune responses [132].

Treatment of Allergic Conjunctivitis

Preventive environmental measures are useful, but not sufficient for the complete control of signs and symptoms of allergic conjunctivitis. A change of climate, especially a move to high mountains during the critical months, avoiding exposure to non-specific triggering factors can provide significant relief to patients. Anyway, the pharmacological therapy in chronic subtypes is usually needed.

Vasocostrictors

The alpha-adrenergic agonists are used topically to control the conjunctival redness. They are non-specific and not pharmacologically active in the cascade of events that leads to the allergic reaction. Moreover, they may also cause side effects such as follicular conjunctivitis, lacrimal punctual occlusion and systemic hypertension, in view of their abuse by chronic allergy sufferers [133].

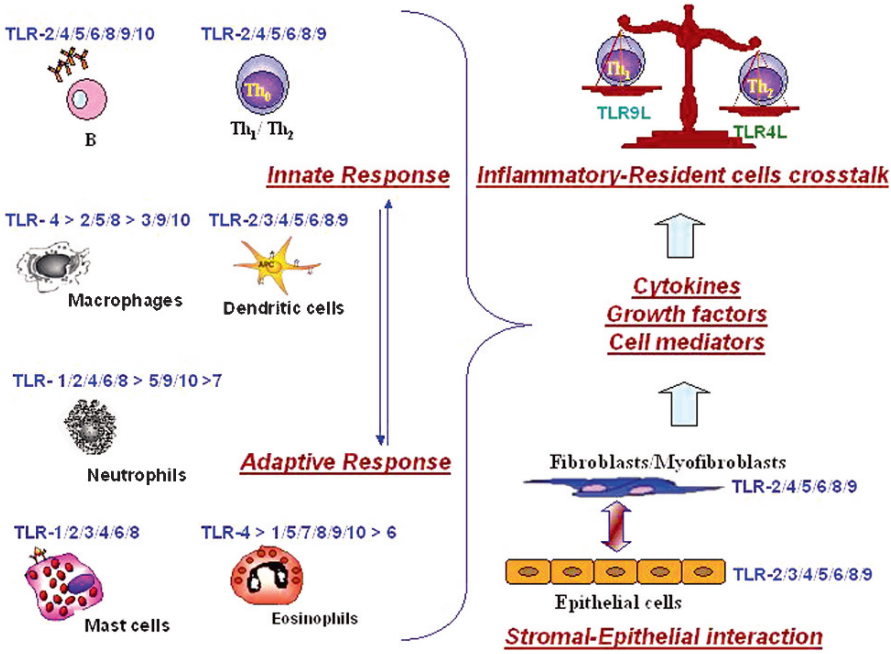

Fig. 2 TLR expression in inflammatory and resident cells. TLR are involved in the cross-talking between innate and adaptive immunity and between immune and resident cells during inflammatory reactions

Antihistamines

Antihistamines are the first line of treatment in ocular allergy, acting as H1-receptor competitive agonists. The new antihistamines have a longer duration of action (4–6 h) and are better tolerated. The second generation topical antihistamines (Cetrizine, Ebastine, Loratadine) offer the same efficacy of their predecessors, but with a low sedative effect and lack of anticholinergic activity. Moreover, for several of these new drugs an inflammatory effect beyond the antihistamine one is reported. In fact, these drugs attenuate the early phase and some features of the late phase of allergic ocular response, above all swelling and redness.

Levocabastine and Emedastine blocked IL-8 and IL-6 release from conjunctival epithelial cells and fibroblasts [134, 135].

Mozolastine is a new H-1 antihistamine with anti-inflammatory properties, developed for treatment of allergic conjunctivitis. Its high efficacy in relieving SAC and PAC is due to the inhibition of the production and release of histamine involved in the late phase of allergic response [136].

EV-131 is a new compound that binds free histamine, stabilizes mast cells, inhibits vascular adhesion molecule expression, and blocks neutrophil and eosinophil chemotaxis.

Finally Transilat, a drug used for keloid, shows potential application in the future in ocular allergic disease. In fact, its inhibitory action on mediator release by mast cells and basophils seems to stop collagen synthesis by fibroblasts.

Mast Cell Stabilizers

This class of drugs inhibits degranulation from mast cells by interrupting the cross-linking and activation of FceRI. All these membrane stabilizers act on the release of histamine and of mediators derived from the arachidonic acid cascade.

Cromolyn sodium was the first molecule studied. It partially inhibits cell degranulation and histamine release [137]. This preventive effect may explain the modest efficacy of these drugs in the clinical treatment of ongoing ocular allergy.

Nedocromil is more potent than cromolyn. It stabilizes conjunctival mast cells and possibly inhibits eosinophils. These drugs are approved for seasonal and perennial allergic conjunctivitis, even if Lodoxamide [138, 139] has been available for VKC.

In addition, it has been shown that N-acetyl aspartyl glutammic acid [139] and Pemirolast alleviate the signs of allergic conjunctivitis [140]. Dipeptide N-acetyl aspartyl glutammic acid 6% is used in Europe as topical eye drops in VKC and GPC because it inhibits leukotriene synthesis, histamine release by mast cells and complement derived anaphylatoxin production [141].

Dual-Action Anti-allergic Drugs

These drugs, at the same time, inhibit histamine release from mast cells and histamine binding to H1 receptors. The advantage is the rapid relief of symptoms given by immediate histamine receptor antagonism (which alleviates itching and redness) and the long-term benefits of mast cell stabilization.

Olapatadine is effective in perennial and seasonal conjunctivitis and allergic symptoms associated with contact lens wear [142]. Ketotifen inhibits release of mediators from mast cells, basophil and neutrophils. It also inhibits PAF production by neutrophils and eosinophil chemotaxis [143, 144].

Azelastine reduces ICAM-1 expression on conjunctival epithelium and inflammatory cell infiltration [145].

Epinastine is a new generation drug with no effect on muscarine receptors [146].

Nonsteroidal Anti-inflammatory Drugs

Ketorolac, a COX inhibitor, acts by blocking the synthesis of prostaglandins, particularly PGD2, which is known to produce significant and immediate allergic symptoms. However, its clinical efficacy seems inferior to olopatadine [147].

Corticosteroids

Corticosteroids should be the last choice in treating allergic diseases, although their use is sometimes unavoidable in VKC and AKC. In fact they may induce the development of cataracts, glaucoma, infections and corneal melting. Fluorometholone can reduce the signs and symptoms of VKC including tearing, discharge, conjunctival redness, papillary hypertrophy and Trantas dots [148].

Anti-leukotrienes

Oral Montelukast demonstrated its efficacy in a pilot study on patients affected by asthma and vernal conjunctivitis in reducing signs and symptoms of ocular allergy after 15 days of treatment [149].

Anti-IgE

Human IgE pentapeptide (HEPP), a synthetic antiallergic agent, which has been under investigation for many years, is thought to competitively block the binding of IgE to cell receptors [150].

Omalizumab, a human recombinant non-anaphylactogenic antibody, is directed against the receptor binding domain of IgE. This binding is specific to free IgE, so IgE is unable to interact with the FceRI on the cells, thereby preventing the antibody from attaching to the mast cell [151]. The use of omalizumab may represent an interesting, still not tested, option for the most severe forms of ocular allergy.

Adhesion Molecule Inhibitors

Adhesion molecule inhibitors may have a role in the treatment of chronic disease with a significant late-phase component, such as VKC or AKC. Natalizumab is a monoclonal antibody to α4-integrin that selectively blocks the VLA-4, which is critical for lymphocytes and eosinophil to adhere to endothelial cells before extravasation [11]. The reported potential side effects of these drugs seem to discourage their use, although, in very severe forms of ocular allergy.

Chemokine Inhibitors

Bertilimumab (CAT-213) is a human IgG4 monoclonal antibody against eotaxin-1, still under development. It is able to inhibit the activation of both the early and late phases of inflammation in murine models of ocular allergy [152].

Immunomodulators

Cyclosporin and Tacrolimus are effective treatments for VKC and AKC [153]. They block cell proliferation and inhibit histamine release from mast cells through the inhibition of calcineurin, a phosphate that plays a key role in the FceRI-mediated exocytosis of pre-formed mediators from mast cell. NFAT, a transcriptor regulator for the production of inflammatory cytokines, is regulated by calcineurin [154, 155]. Cyclosporin and Tacrolimus block the release of NFAT-mediated cytokines from T-lymphocytes and mast cells, reduce eosinophil infiltration and decrease cellular adhesion to the site of inflammation. However treatment with these drugs may be at risk of folliculitis, acne and herpes simplex [156].

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Allergy Frontiers: Clinical Manifestations
Pawankar, R.; Holgate, S.T.; Rosenwasser, L.J. (Eds.)
2009, XXII, 511 p., Hardcover
ISBN: 978-4-431-88316-6