



The ocular surface: from physiology to the ocular allergic diseases

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RESUMEN

La conjuntivitis alérgica es la inflamación de la conjuntiva secundaria a una respuesta inmunitaria contra antígenos exógenos, usualmente llamados alérgenos. De hecho, la conjuntivitis alérgica es un síndrome que involucra la totalidad de la superficie ocular, incluyendo la conjuntiva, los párpados, la córnea y la película lagrimal. Los signos y síntomas de la conjuntivitis alérgica tienen un efecto significativo en el bienestar y salud del paciente y pueden ser influidos por el ambiente, la genética y mecanismos de regulación inmunológicos, todos los cuales trabajan en conjunto en una compleja homeostasia inmunológica. La disregulación de estos mecanismos puede desembocar en una gran variedad de enfermedades alérgicas oculares. Esta revisión describe algunos de los conocimientos celulares y moleculares actuales, involucrados en las diferentes enfermedades alérgicas oculares.

Palabras clave: conjuntivitis alérgica, enfermedades alérgicas oculares, Tregs, linfocitos T CD4+.

ABSTRACT

Allergic conjunctivitis (AC) is an inflammation of the conjunctiva secondary to an immune response to exogenous antigens, usually called allergens. In fact, AC is a syndrome that involves the entire ocular surface, including conjunctiva, lids, cornea, and tear film. The signs and symptoms of AC have a meaningful effect on comfort and patient health, and could be influenced by environment, genetics and immune regulation mechanisms, all of which work together in a complex immunological homeostasis. Dysregulation in such immune responses could turn into a variety of ocular allergic diseases (OAD). This review describes some of the current understanding of cellular and molecular pathways involved in different OAD.

Key words: allergic conjunctivitis, ocular allergic diseases, Tregs, CD4+ T cells.

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Allergies affect up to 30-40% of the population worldwide, and the severity and complexity of allergic diseases has been increased in the last years. Globally, 300 million people suffer from asthma, approximately 200-250 million people suffer from food allergies, one tenth of the population suffers from drug allergies, and 400 million suffers from rhinitis.¹ In México, according to the Instituto Nacional de Estadística y Geografía (INEGI, all types of conjunctivitis are within the first ten causes of morbidity in our population;² and allergic conjunctivitis (AC) is the second cause of eye care, at the Ophthalmology Institute Fundación Conde de Valenciana, a national ophthalmologic referral center, with more than 9,000 ophthalmic consultations per year only for AC. Thus, AC is one of the most common eye disorders in the daily ophthalmological clinical practice.

Definition and clinical classification

Allergic conjunctivitis has been defined as an ocular surface disease secondary to dysregulation of the immune system that involves bilateral conjunctival inflammation.⁴ Allergic conjunctivitis is considered a syndrome that comprises the entire ocular surface, including conjunctiva, lids, cornea, and tear film.⁵⁻⁷ The symptoms include itching, gritty or burning sensation, tearing and light sensitivity; however, if antigenic stimulation continues, irreversible changes would appear on the ocular surface as a result of the immune response and tissue healing mechanisms (See Immunological mechanisms of ocular allergic diseases in this minireview).

Allergic conjunctivitis includes a spectrum of a number of traditional overlapping ocular allergic diseases (OAD) that range from intermittent to persistent signs and symptoms, all of them variable in severity and presentation, thus clinical diagnosis is still a challenge. Allergic conjunctivitis could present as mild forms with transient inflammation, such as seasonal (SAC) and perennial allergic conjunctivitis (PAC), or as more severe persistent and chronic inflammatory forms such as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC).^{Reviewed in 5-9}

Due to these overlapping conditions, we developed a grading score to evaluate objectively the clinical severity of OAD⁵ (Figures 1-2 and Table 1). This classification corresponds to numerous signs and symptoms following a grade of severity; the total possible score is 48 points, twenty of them corresponding to symptoms, (Figure 1) and twenty eight to signs (Figures 2 and Table 1).

The clinical grade of severity is defined as follows: 0 points: absent, 1-12 points: (mild), 13-24 points: moderate, 25-36 points: moderately severe and 36-48 points: severe. The final score is useful in recognizing the progress of ocular allergic disease, and could be used as an objective clinical score, in clinical research, and throughout therapeutic interventions (reviewed in 8). The characteristics of SAC and PAC patients might correspond mainly to grade 1 and 2; the characteristic signs of VKC patients might correspond to grade 2 and mainly grade 3; and ocular signs of AKC patients might correspond to grade 3 and mainly grade 4.

	0 None of the time	1 Some of the time	2 Half of the time	3 Most of the time	4 All of the time
Itching					
Tearing					
Light Sensitivity					
Gritty Sensation					
Burning Sensation					

Figure 1. Grade of severity, symptoms of allergic conjunctivitis. Figure from Robles-Contreras, Santacruz C, et al. This figure have a Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The ocular surface in non-inflammatory conditions

The ocular surface is a functional unit essentially formed by the conjunctival, limbal, and corneal epithelium (structural component); and by the tear film (soluble component).

Conjunctiva

The conjunctiva is a lining of the outer portion of the eye. Conjunctival tissue begins from the anterior portion of the limbus, and ends at the eyelids margin. Anatomically, conjunctiva is divided into three regions: *i*) the bulbar conjunctiva, which covers the anterior portion of the sclera; *ii*) the palpebral conjunctiva, which lines the inner surface of the eyelids; and *iii*) the conjunctival sac or fornix, which is the space bounded by the bulbar and palpebral conjunctiva.⁹

Histologically, is divided into 2 layers: epithelium and lamina propria. The epithelial layer is composed of 2 to 5 cells of non-keratinized stratified columnar cells with mucous secreting goblet cells, while the lamina propria is composed by richly vascularized connective tissue. In humans, intraepithelial leukocytes are T cells and dendritic cells expressing the human mucosal lymphocyte antigen (HML-1).¹⁰ HML-1 or CD103 is an adhesion molecule (aEb7 integrin) that was first described as a molecule related to mucosal migration. Under physiological conditions, conjunctival epithelium does not contain any inflammatory cells such as polymorphonuclear (PMN) cells, eosinophils, basophils or mast cells; these cells are commonly found in the lamina propria, the layer just below the epithelial surface. (Figure 3)



Figure 2. Grade of severity, ocular signs of allergic conjunctivitis. Figure from Robles-Contreras, Santacruz C, et al. This figure has a Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The conjunctival vessels are born from the conjunctival sac, continuing through the bulbar conjunctiva, into their superficial and deep layers; at that point, the vessels are directed to the limbus, to finish anastomosing with the deep vessels at sclera. The conjunctival lymphatic drainage is divided into nasal and temporal. The nasal portion of conjunctiva drains into the submental lymph nodes; while the temporal portion of conjunctiva, drains into preauricular lymph nodes.

T, B and accessory cells are distributed diffusely or in organized structures at lamina propria, these immune cells are part of conjunctiva associated lymphoid tissue (CALT). CALT is part of the mucosa-associated immune system that is extended from ocular surface (conjunctiva and cornea) along with its mucosal adnexa (the lacrimal-drainage-associated lymphoid tissue, LDALT),

all together this immune surveillance system constitutes the eye associated lymphoid tissue (EALT).¹¹ Regarding conjunctival innervation, sensory fibers derived from trigeminal nerve can contribute to the allergic inflammatory response releasing neurotransmitters to the ocular microenvironment (see Neurogenic Inflammation as Promoting Factor in Ocular Allergy section in this minireview).

Cornea

Cornea is a transparent avascular tissue, divided into six layers: the epithelium, Bowman's membrane, the stroma, Dua's layer, Descemet's membrane and endothelium.¹²

Corneal epithelium is stratified, squamous and non-keratinized. The ability of the ocular surface to begin an immune response is partly attributed to proteins called

Table 1. Grade of severity, ocular signs of AC. Figure from Robles-Contreras, Santacruz C, et al. This figure have a Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

Grades	Eyelid position and skin aspect	Eyelid margin Marx's Line (MGD)	Signs			Tarsal conjunctiva inflammation response	Limbus involvement	Cornea involvement
			Conjunctiva hyperemia and swelling	Conjunctiva discharge	No discharge			
0	No eyelid edema	No displacement of MCJ	No hyperemia or edema	No discharge	No discharge	No papillary hyperplasia or visible follicles	No visible limbus nodules or dots	No SPK
1	Localized superior or inferior eyelid margin edema without Dennie Lines	1/3 displacement of MCJ inferior or superior eyelid margin	Hyperemia 1+ - 2+ with 1/3 pink edema aspect in conjunctiva. No conjunctiva plica formation in sac fundus	Clear watery discharge and/or slight debris within	Clear watery discharge and/or slight debris within	Less than 1/3 tarsal papillae size 0.3 with visible uniform conjunctiva tarsal vessels	Less than one quadrant with dot trantas	Slight SPK without central involvement
2	Generalized superior and/or inferior eyelid edema with slight pseudoapoptosis and Dennie Lines	2/3 displacement of MCJ inferior or superior eyelid margin	Hyperemia 2+ -3+ with 2/3 redness edema aspect in conjunctiva, and/or slight conjunctiva plica formation in sac fundus	White-gray mucoid discharge in sac fundus or adherent to limbus or tarsal conjunctiva	White-gray mucoid discharge in sac fundus or adherent to limbus or tarsal conjunctiva	1/3 to 2/3 moderate tarsal papillae 0.3-0.5 size with thin visible tarsal conjunctiva vessels	One 1/4 to one half of dots trantas on limbus with slight pigment	One quarter to one half of SPK without compromise of visual axis
3	Unilateral or bilateral moderate pseudoapoptosis and several Dennie Lines	Generalized displacement of MCJ	Hyperemia > 3+ with more than 2/3 conjunctiva edema with localized engorgement of ciliary vessels. Moderate plica formation in sac fundus	White, gray or yellow thick copious mucoid strands in sac fundus or adherent to limbus of tarsal conjunctiva	White, gray or yellow thick copious mucoid strands in sac fundus or adherent to limbus of tarsal conjunctiva	Cobblestone papillae presentation. More than 2/3 tarsal papillae 0.75 size, with or without fibrosis, fairly irregular tarsal vessels	More than one half of dots trantas on limbus with slight to moderate pigment or 1/4 to one half of LSCD	Generalized SPK with compromised epithelial defects. Indolent corneal ulcer on superior quadrants
4	uni or bilateral severe pseudoapoptosis with Dennie Lines and changes on skin texture and pigmentation. Hertoghe's sign present	Scarring or keratinized changes	Same as grade 3+ generalized engorgement of ciliary vessels. Severe plica or conjunctiva folding formation in sac fundus	Thin copious farely strands adherent mainly to cornea surface	Thin copious farely strands adherent mainly to cornea surface	Few tarsal papillae > 0.75 with fibrosis or macro papillae extrusion and possible fornix foreshortening (symblefaron) or generalized pale tarsal conjunctiva aspect without visible tarsal vessels	Generalized dot trantas on limbus with fibrosis and pigment or more than one half of LSCD	Keratoconus with or without central leucoma

Hyperemia grading. 0 = absence of hyperemia; 1+ = mild (1/3 localized sector engorgement of bulbar conjunctival vessels), 2+ = moderate (2/3 diffuse engorgement of bulbar conjunctival vessels), 3+ = severe (significant generalized engorgement of bulbar conjunctival vessels).

SPK: superficial punctate keratopathy; LSCD: limbal stem cell deficiency; MGD: meibomian gland disease; MCJ: mucocutaneous junction.

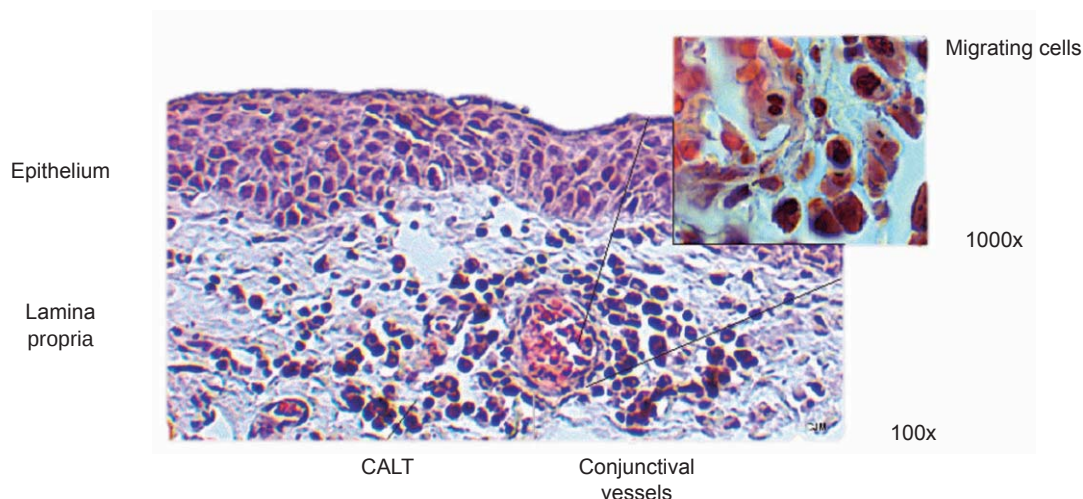


Figure 3. Healthy human conjunctiva. Histological components of healthy human conjunctiva are shown (Haematoxylin and eosin stain, 100x). Upper right, conjunctival vessels are observed at 1000x magnification. CALT: conjunctiva associated lymphoid tissue.

pattern recognition receptors (PPR) that are expressed on epithelial cells from cornea and conjunctiva. (Table 2)

Toll like receptors (TLR) are the most studied PPR at ocular surface; TLR are type I transmembrane proteins

Table 2. Pattern recognition receptors in ocular surface

Receptor	Ligand	Location in ocular surface
TLR1	Lipoproteins	Corneal epithelium and stroma. Conjunctival epithelium ^{13,14,75}
TLR2 (TLR1)	Tryacil lipopeptids, glycolipids, lipoproteins, PGN, LTA, zymosan, LTA, mycobacterial lipoarabinomannan, zymosan (β -1,3-glucan and β -1,6-glucan) and heatshock protein 60	Corneal epithelium and stroma. Conjunctival epithelium ^{13,14,76}
TLR3	dsRNA viruses (double-stranded)	Corneal epithelium and stroma. Conjunctival cells ^{13,14,77}
TLR4	LPS, glycoinositolphospholipids, heat-shock proteins, fibrinogen, hyaluronic acid, b-defensin and extracellular domain A in fibronectin	Corneal epithelium and stroma. Conjunctival epithelium and stroma ^{13,14,78,79}
TLR5	Flagelin	Corneal epithelium and stroma. Conjunctival epithelium and stroma ^{13,14}
TLR6 (TLR2)	Dyacil lipopeptids, zymosan, lipoteichoic acid and peptidoglycan	Corneal epithelium and stroma. Conjunctival epithelium ^{13,14,80}
TLR7	ssRNA viruses (single strand), synthetic antiviral imidazoquinoline compounds such as R848, loxoribine and imiquimod	Corneal epithelium and stroma. Conjunctival epithelium ^{13,14,81}
TLR8	ssRNA viruses (single strand), synthetic antiviral imidazoquinoline compounds such as R848, loxoribine and imiquimod	Corneal epithelium ^{13,14,82}
TLR9	Unmethylated CpG motifs of ssDNA (bacteria and virus)	Corneal epithelium and stroma. Conjunctival epithelium and stroma ^{13,14,83}
TLR10	ND	Corneal epithelium ^{13,14}
NOD1	iE-DAP	Corneal epithelium ^{84,85}
NOD2	Muramyl dipeptide	Corneal epithelium. Limbal fibroblasts ^{86,87}

TLR: Toll like receptor; PGN: peptidoglycan; LTA: lipoteichoic acid; LPS: lipopolysaccharide; ND: not determined; iE-DAP: γ -D-glutamyl-meso-diaminopimelic acid; NOD: nucleotide oligomerization domain.

with extracellular leucine-rich domain and intracellular domains called TIR (Toll/IL-1 domain receptor). TLR are able to recognize microbial pathogens and to trigger the immune response leading to inflammation, through production of cytokines, chemokines and increasing expression of adhesion molecules.¹³ It has been suggested that due to corneal and conjunctival epithelial cells are in constant contact with bacterial microbiota and their products, TLR expression at ocular surface is highly regulated; thus, some mechanisms have been reported to avoid unnecessary immune activation, i.e. TLR4 and TLR5 are expressed at basal and wing cell layers, but not at the apical layers of the corneal epithelium.¹⁴ Other authors have reported that despite epithelial cells are expressing TLR4, neither corneal cells nor limbal cells are able to produce proinflammatory cytokines.^{15,16}

Functional activation of TLR promotes adaptive immune response by increasing expression of MHC class II molecules, as well as higher expression of costimulatory molecules on antigen presenting cells resident in the stroma of cornea and conjunctiva. However, immune activation may result in further damage to the visual function.^{13,17}

Like TLR, nucleotide binding oligomerization domain receptors (NOD)-like receptors (NLR) are involved in recognition of bacterial stimuli. After activation, NLR and TLR have been implicated in human beta-defensin (HBD) expression at ocular surface. HBD have a wide range of functions from antimicrobial effect, immune modulation, to crosstalk between innate and adaptive immunity. HBD are up- or down- regulated in a time-dependent manner in response to several TLR or NLR ligands (Table 1),^{18,19} therefore, more research is needed to understand the involvement of TLR and NLR at ocular surface in both, normal and pathological conditions.

Corneal limbus

Limbal epithelial cells (LEC) are stem cells located at the intermediate zone between the corneal crown and the scleral brim. LEC give rise to the corneal epithelium, and limbal cells are also responsible for corneal epithelial tissue repair and complete regeneration after injury. LEC are able to release antiangiogenic and proangiogenic growth factors in a delicate balance; if this stability is broken by inflammation, it is possible neo-vascularization of the ocular surface, including cornea.²⁰

The tear film

The tear film is a trilaminar barrier that protects ocular surface. Lipids secreted by Meibomian glands constitute the outer layer. The lipid layer lubricates the eyelid and reduces evaporation of the aqueous tear film layer. The middle layer is the aqueous layer and represents 98% of the tear film. The main lacrimal gland and the accessory lacrimal glands are producing the aqueous layer, which contains several antimicrobial proteins, secretory IgA, and complement proteins. The inner layer is composed by secreted mucins and membrane-bound mucins. Goblet cells of conjunctiva are the main source of secreted mucins, mainly MUC2, MUC5AC, and MUC19;²¹ while membrane-bound mucins includes MUC1, MUC4 and MUC16.²² The mucin layer together with the aqueous layer comprises a muco-aqueous functional unit.²³

Immunological mechanisms of ocular allergic diseases

Two stages have been defined in AC as immunological mechanisms of ocular allergy. The first stage is named sensitization phase reaction, and is initiated by preferential activation and polarization of the immune response to environmental antigens, that culminates with a generation of a predominant Th2 immune response and production of IgE antibodies; and the second stage, named effector phase reaction, which is initiated with a second encounter with antigen (Ag) leading to activation of effector mechanisms, such as degranulation of granulocytes and release of diverse inflammatory mediators.

Sensitization phase reaction

Bronchial and nasal mucosa, have the ability to capture Ag through Langerhans cells (LC), as it has been reported in patients with asthma and allergic rhinitis,^{24,25} LC process and presents Ag in the context of MHC-II molecules and stimulate specific CD4+ T cells to induce secretion of IL-4, IL-13 and expression of CD154; this process activates genetic recombination in B cells and class switching to IgE. Similar mechanisms could be involved in ocular mucosa, since it has been reported that total and specific IgE could be detectable in human tears from SAC, PAC, and VKC patients, and correlates with active allergic conjunctivitis.^{26,27} Similarly, in VKC

patients it has been reported an increased frequency of B cells expressing CD23, CD21, and CD40, suggesting that they might be activated B cells, and local precursors of IgE.²⁸ Interestingly, mast cells and basophils from giant papillae biopsies obtained from AKC and VKC patients are hyperexpressing the high-affinity IgE receptor (Fc ϵ RI) than mast cells from healthy conjunctiva.²⁹

Effector phase reaction

Allergen-induced cell degranulation is the key event in allergic inflammation and leads to two effector phases: a) Early phase reaction, and b) Late phase reaction.

Early phase reaction (EPR). The second encounter with the antigen on IgE-sensitized cells, induces the cross-linking of their receptors: Fc ϵ RI, or Fc ϵ RII (CD23). Cross-linking of IgE receptors provokes: a) the release of preformed mediators such as histamine, a vasoactive amine; proteases, such as chymase and tryptase; and chemotactic factors, such as eosinophil cationic protein (ECP), b) activation of transcription factors and cytokine gene expression, and c) production of lipid mediators (prostaglandins and leukotrienes) by the phospholipase A2 pathway.⁵

EPR and preformed mediators. Histamine is a monoamine released by sensitized mast cells upon exposure to allergen, the released histamine binds to their receptors located at the endothelium, neuronal fibers, and conjunctival epithelium resulting in the cardinal signs and symptoms of ocular allergy: itching, erythema, tearing, chemosis, and palpebral oedema. Histamine receptors (H1, H2, H3 and H4) are expressed on goblet cells, and goblet cell secretion is induced after receptor stimulation.³⁰ Histamine secretion may also recruit immune cells that cause long-term damage to the ocular surface;³¹ and an increased expression of H4 receptors has been reported in inflammatory cells from conjunctiva in VKC patients.³²

Activation of mast cells by IgE is relevant since it is well known that there are up to 6000 mast cells/mm³ in conjunctiva,³³ and mast cell density is increased in SAC and AKC patients.^{34,35} Two types of mast cells (MC) subsets have been described, MC chymase+ and MC tryptase+. Remarkably, these two MC subsets are important source of IL-4, IL-5, IL-6 and IL-13 in patients with SAC,³⁶ and are involved in the pathogenesis of AKC,

and VKC^{37,38} through activation of matrix metalloproteinases (MMP).^{39,40} ECP is a ribonuclease, which has been involved in several allergic diseases as a cyto- and neurotoxic mediator; ECP is also a promoting fibrosis factor, largely recognized in rhinitis and asthma.⁴¹ In addition, ECP has been implicated as a soluble mediator at the beginning of the early phase reaction in pollen-induced ocular allergy, and also in patients with SAC, PAC, VKC and AKC.⁴²⁻⁴⁵

Late phase reaction (LPR). Cellular infiltration is the main feature of the LPR, and begins 4-24 h after EPR;⁴⁶ once initiated, LPR can proceed even without allergen-specific IgE antibody.

In the chronic forms of AC, allergen mediated inflammation is maintained by infiltrating CD4+ T cells to conjunctiva;⁴⁷ and migration of effector cells, such as eosinophils, basophils and also T cells is dependent on eotaxin-CC-chemokine receptor (CCR)-3 expression. Notably, CCR3 chemotaxis induced by culture supernatant from corneal keratocytes and tear samples from severely allergic patients could be inhibited by specific monoclonal antibodies against CCR3.⁴⁸ Recently, we demonstrated that in patients with PAC an increased frequency of circulating activated CD4+ T cells, expressing CCR4 and CCR9, and decreased frequency of CD4+CD25+FOXP3+ cells (Tregs)⁴⁹ CCR4+ cells are important source of IL-4, IL-5 and IL-13;^{50,51} on the other hand, CCR9 is a molecule expressed on antigen-experienced memory T cells, and is a surface marker related to mucosal homing;⁵² remarkably IL-4 is required for CCR9 imprinting on CD4+ T cells.⁵³ Interestingly, after *in vitro* allergenic-specific stimulation the frequency of CD4+CCR4+CCR9+ was increased, with production of IL-5, IL-6 and IL-8. These data suggest that interaction of CCR4 and CCR9 with their ligands on conjunctiva, favours the selective adhesion of a circulating activated CD4+ T cell subsets, driving the immune-response to the ocular mucosa and inducing a proinflammatory Th2 microenvironment;⁴⁹ In line with this, it is well known that IL-6 is a cytokine that inhibits Treg differentiation;⁵⁴ thus, the lower frequency of Tregs observed in peripheral blood from PAC patients could be associated with higher production of IL-6. Low percentage or dysfunction of Tregs, reinforces disease progression, as it has been recently described in asthma.⁵⁵

The active phase of chronic inflammation is characterized by multiple Th1-type and Th2-type cytokines that are over expressed on ocular surface. The Th1-type cytokines, interferon (IFN)- γ and the pro-inflammatory TNF- α , might influence the delayed hypersensitivity ocular damage, as suggested in VKC and AKC patients.^{56,57} In addition, IFN- γ , TNF- α and IL-4 can modulate the TGF- β signaling pathway favoring tissue remodelling by conjunctival fibroblast in VKC;⁵⁸ while IL-1b, IL-4

and TGF- β induce VEGF, promoting neovascularization and giant papillae formation in AKC and VKC.⁵⁹ This particular microenvironment, enriched with TNF- α and IFN- γ , is also involved with changes in mucins expression,⁶⁰ as it has been demonstrated in AKC patients. The conjunctiva from AKC patients shows up-regulation of MUC1, MUC2 and MUC4, and down regulation of MUC5AC, this altered profile could contribute to the damage at ocular surface.^{61,62} (Figure 4)

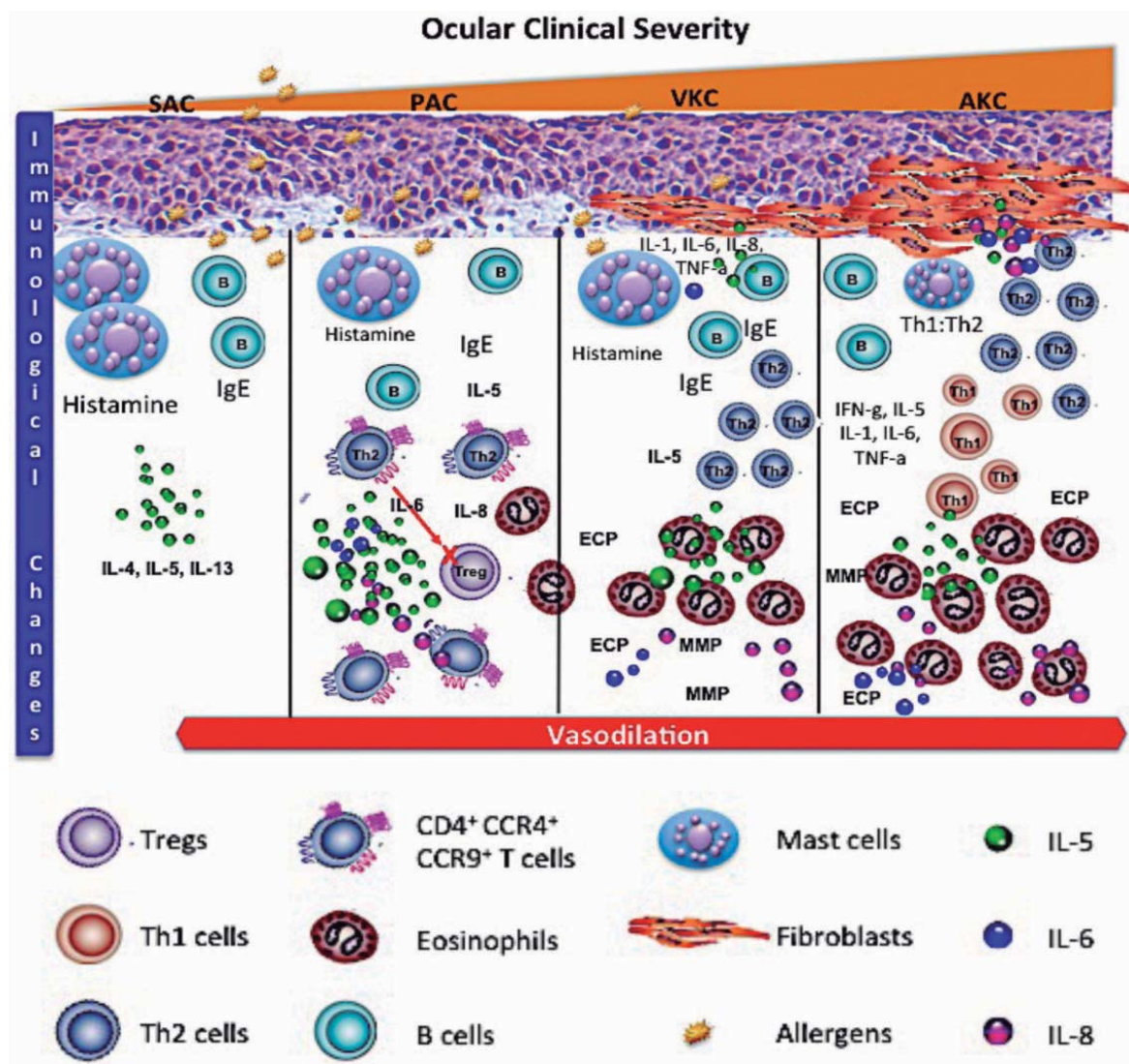


Figure 4. Immunological mechanisms of ocular allergic diseases, and clinical severity. Schematic immunological changes observed in ocular allergic diseases. SAC: seasonal allergic conjunctivitis; PAC: perennial allergic conjunctivitis; VKC: vernal keratoconjunctivitis; AKC: atopic keratoconjunctivitis; ECP: eosinophil cationic protein; MMP: matrix metalloproteinases; IFN: interferon; TNF: tumor necrosis factor. Modified from reference 63.

Neurogenic inflammation as promoting factor in ocular allergy

The immune system and nervous system are closely interconnected through innervation of lymphoid organs and/or with soluble molecules from neural- or immunological source, and receptors on target cells from both systems. Ocular surface innervation is provided by a relatively small number of primary sensory neurons located at the ipsilateral trigeminal ganglion. The majority of sensory fibres (about 70%) are polymodal nociceptors, that are equally activated by a near-noxious mechanic stimulus and also are able to respond to different triggers like heat, exogenous chemical irritants, and endogenous soluble mediators released by resident inflammatory cells or from plasma leaked since limbal capillary vessels (i.e. cytokines, growth factors, kinins, prostaglandins and arachidonic acid metabolites).⁶⁴

Under physiological conditions, primary sensory fibres are in a silent state, but after tissue damage, i.e. acidosis or temperature changes, vanilloid transitional type 1 (TRPV1) receptors located at the nerve endings are stimulated. After TRPV1 activation, a variety of neuropeptides are released from sensory fibres, mainly substance P (SP) and the calcitonin gene related peptide (CGRP); these neuropeptides activate a wide variety of signalling cascades, all of them involved with inflammation, oedema and pain.^{65,66} Nociceptor antidromic stimulation contributes synergistically to propagate still more the inflammatory response; and the wide range of triggers induce more receptors on sensory fibres that are able to sense more stimuli, a process called sensitization. The hyperactivation of sensory fibres causes vasodilatation, plasma extravasation and decreased pain threshold.⁶⁷ The set of mechanisms that result in the activation and sensitization of primary sensory fibres is called neurogenic inflammation. The importance of neurogenic inflammation in the ocular surface is suggested by the large trigeminal sensory innervation in cornea and conjunctiva; trigeminal innervation is involved in neurogenic inflammation in animal models of migraine, and clinical studies;⁶⁸⁻⁷⁰ the involvement of TRPV1 in allergic processes has been recently demonstrated in asthma.⁷¹ Thus, neurogenic mechanisms may have a significant role in the onset and chronicity of ocular surface inflammation; there is

increasing evidence that these fibres can release different neuropeptides in ocular microenvironment in OAD. SP, CGRP, and VIP are induced after conjunctival challenge by specific allergens in non-active allergic conjunctivitis patients,⁷² and elevated levels of SP in tears, and VIP in conjunctival tissue have been reported in VKC patients.^{73,74} Nevertheless, the studies about involvement of neurogenic inflammation in the course of OAD are still insufficient to understand how nervous system and the immunological system interact to develop health or disease at the ocular surface.

Conclusions and future approaches

Ocular allergic diseases have become a special concern for medical specialists, the clinical diagnosis is still a challenge due to a wide range of overlapping entities which might respond differently to conventional treatments; then, in order to understand the neuro- and immunological mechanisms involved in OAD, and to develop new perspectives in the treatment of the most frequent ocular condition seen by allergo/immunologists and ophthalmologists, more research is needed from the basic view to clinical applications.

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