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REVIEW ARTICLE

Epithelial barrier dysfunction in ocular allergy

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Abstract

The epithelial barrier is the first line of defense that forms a protective barrier against pathogens, pollutants, and allergens. Epithelial barrier dysfunction has been recently implicated in the development of allergic diseases such as asthma, atopic dermatitis, food allergy, and rhinitis. However, there is limited knowledge on epithelial barrier dysfunction in ocular allergy (OA). Since the ocular surface is directly exposed to the environment, it is important to understand the role of ocular epithelia and their dysfunction in OA. Impaired epithelial barrier enhances allergen uptake, which lead to activation of immune responses and development of chronic inflammation as seen in allergies. Abnormal expression of tight junction proteins that helps to maintain epithelial integrity has been reported in OA but sufficient data not available in chronic atopic (AKC) and vernal keratoconjunctivitis (VKC), the pathophysiology of which is not just complex, but also the current treatments are not completely effective. This review provides an overview of studies, which indicates the role of barrier dysfunction in OA, and highlights how ocular barrier dysfunction possibly contributes to the disease pathogenesis. The review also explores the potential of ocular epithelial barrier repair strategies as preventive and therapeutic approach.

KEYWORDS
allergic conjunctivitis, epithelial barrier, glycocalyx, ocular allergy, tight junctions, vernal keratoconjunctivitis

1 | INTRODUCTION

The eye is a complex organ with unique anatomical and physiological barriers including the complex junctions present in the conjunctival and the corneal epithelium, the blood-aqueous barrier, and blood-retinal barrier. These barriers offer protection and limit the exchange between the eye tissues and the external and internal environment. The key function of epithelium is to act as a physical and immune barrier preventing the entry of pathogens and allergens apart from specialized function and tissue homeostasis. The epithelial junctions along with components of innate immune response (antimicrobial peptides, lipids, cytokines, and pathogen recognition receptor systems) provide a protective physical barrier to microbes and environmental insults. Epithelial barrier dysfunction is a hallmark feature of several allergic disorders.[1,2] When this barrier is disrupted, allergens and pathogens gain access to the underlying connective tissue of lamina propria, eliciting a strong innate immune response via pathogen recognition receptors (PRR) present on the epithelial and the immune cells (macrophages, mast cells). The subsequent sensitization and activation of adaptive immune response lead to type 2 inflammation characteristic of the allergic disorders. Barrier disruption can also occur because of defects in several essential structural components of epithelium apart from other known contributing factors such as genetic predisposition, immune and environmental stimuli, and altered microbiome. The role of defective epithelial barrier has been well established in asthma, dermatitis,

rhinitis, and food allergies. However, there are fewer studies available on how ocular surface barrier dysfunction is involved in initiation or exacerbation of ocular allergy (OA). This review provides a comprehensive overview of the studies indicating the role of impaired ocular epithelium in initiation or progression of OA. The review further describes the factors contributing toward the ocular barrier dysregulation in OA and explores the potential of ocular epithelial barrier repair strategies as preventive and therapeutic approach for OA (Box 1).

2 | THE OCULAR SURFACE BARRIERS

The ocular surface consists of conjunctiva, cornea, limbus (delimiting the two of them), lacrimal gland, lacrimal drainage apparatus and associated eyelid structures. Each part of the ocular surface has unique functions such as protection, tear production, and drainage, with predisposition to specific diseases. The ocular surface barrier includes two layers of protection, the transcellular barrier, also called epithelial glycocalyx, on the foremost apical cell membranes,[3] and the paracellular barrier including the stratified structure of the corneal and conjunctival epithelia. In addition, the acellular epithelial basement membrane in cornea serves as a barrier to penetration of cytokines from the epithelium to stroma, and possibly from stroma to epithelium.[4]

2.1 | Glycocalyx barrier

The glycocalyx comprises membrane-associated mucins (MAMs) and galectin-3, which crosslinks to form an interlocking lattice.[5] The membrane folds (microplicae) on the ocular surface epithelium are largely made up of glycoproteins called mucins (MUC) that forms the boundary between epithelium and the tear film. On ocular surface, mucins exist in secreted form and MAMs. The secreted mucins are synthesized by conjunctival goblet cells, forms the bulk of mucus and remain in constant motion over epithelial surfaces. MAMs and their O-glycans form a continuous glycocalyx on the apical side of the epithelia and constitute a protective barrier preventing the penetration of extracellular agents.[5,6] Of the several MAMs identified, the ocular surface epithelium consists primarily of MUCs 1, 4, and 16.[7,8] Corneal and conjunctival epithelia express MUC1 and MUC16, while conjunctival epithelium express MUC4.[8] N-glycans and O-glycans guarantee the cross-linked interaction between MAMs and galectin-3 on the apical cells necessary preserve the epithelial barrier integrity, prevent cellular damage,[9] and infections, and to modulate inflammation. For example, MUC1, being an adhesion receptor for various pathogens, promotes microbial clearance representing a first line of defense against bacterial and viral infections.[10] Further, the glycocalyx pathogen-exclusion function is associated with TLR-mediated innate immune response[11] where MUC1 regulates pathogen-mediated inflammation by inhibiting TLR signaling and NLRP3-inflammasome activation.[12]

BOX 1 Key highlights of the review

- The cornea and conjunctiva, even though anatomically and physiologically connected have altogether different barrier functions, junctional apparatus and interactions with the respective underlying connective tissues.
- The ocular surface, including the corneal and conjunctival epithelia is directly exposed to environmental conditions and influenced by the exposome. Therefore, role of ocular barrier is crucial.
- The non-structured conjunctival associated lymphatic tissue can recognize and processing foreign antigens and initiate a specific immune response.
- Ocular allergy is not a single disease but a collection of different disorders with specific signs and symptoms, phenotypes, prognosis, and management.
- Epithelial defects and tissue remodeling are typical features of chronic OA such as VKC and AKC.

2.2 | Epithelial barrier

To act as a barrier, epithelial cells in mammals are sealed together by three apico-lateral structures, namely, tight junctions (TJs), adherens junctions (AJs), and desmosomes, arranged on the lateral side of the epithelium[13] (Figure 1). TJs, including claudins, MARVEL domain proteins (occludin, tricellulin), and junctional adhesion molecules (JAMs) create a barrier, whereas AJs and desmosomes function in cell-cell adhesion and are generally referred to as apical junctional complex. Gap junctions, on the basolateral side act as bridge between the two cells and play a key regulatory role in cell differentiation and growth.[13]

Of the three key transmembrane proteins common to all TJs, claudins and occludin are two most important proteins. Other important TJ framework proteins are cingulin, Proteins Associated with LIN7 1, Multi-PDZ domain protein 1, and ZO-1, ZO-2, ZO-3.

Allergens, pathogens, and pollutants are known to cleave the TJs between epithelial cells to enter the paracellular space.[14] In allergic diseases, TJs disruption is observed in the epithelial cells of patients with asthma, atopic dermatitis, and rhinitis[15–17] induced by proteases present in pollens or house dust mites, or by cytokines, environmental pollutants such as PM 2.5 and cigarette smoke.[18–20] Although the role of TJs disruption in allergy has been increasingly studied, it is not clear whether protection of the barrier can prevent the allergic disorders.

Conjunctival and corneal epithelia form a physical barrier and protect the eye from the external environment. The conjunctival epithelium is 3–5 cell layer thick, composed of non-keratinized, stratified squamous and stratified columnar epithelium[21] and contains scattered goblet cells that produce mucins, in particular MUC5AC

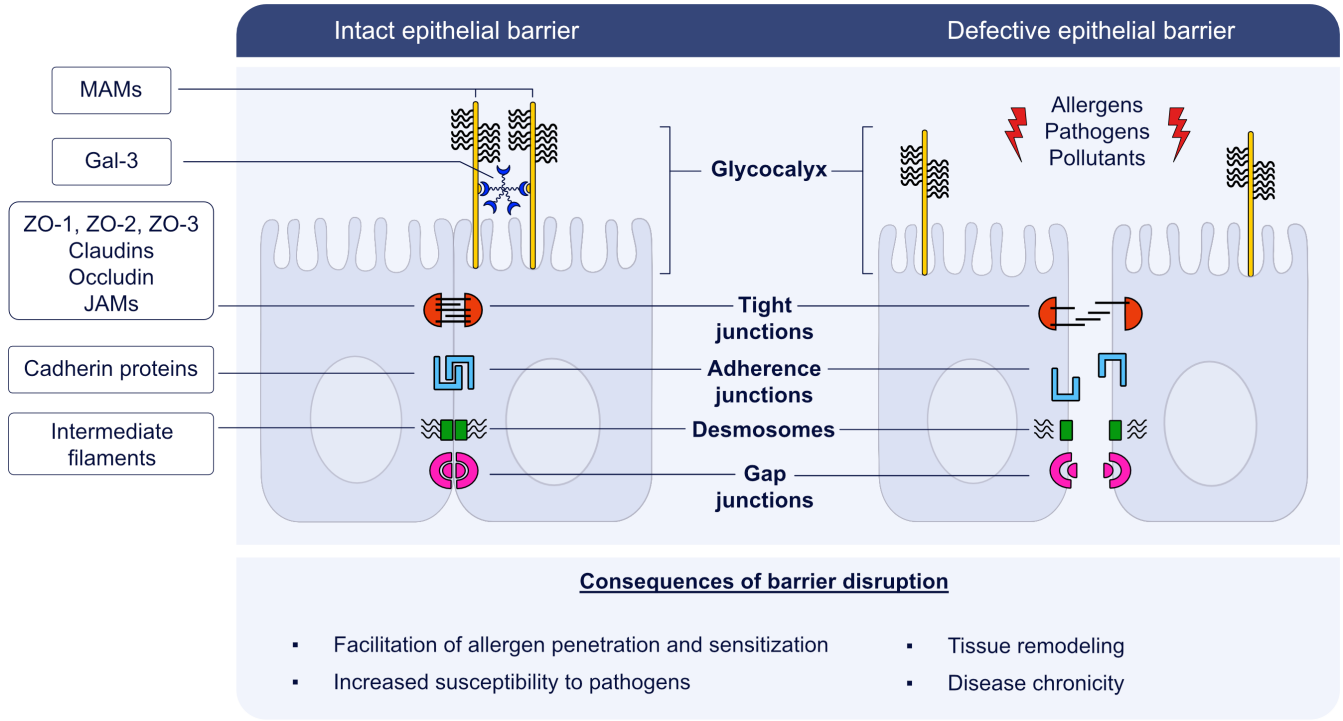

FIGURE 1 Schematic illustration representing the structure of the epithelial barriers in normal ocular surface epithelium. Adjacent epithelial cells, covered by the glycocalyx made up of membrane-associated mucins (MAMs) and galectin-3, adhere to each other through a network of transmembrane proteins: tight junctions (TJs), reside in the apical lateral side and consist of the anchoring proteins, occludin, claudins, and junctional adhesion molecules (JAMs). JAMs are connected to the cytoskeleton through the zonula occludens (ZO)-1, ZO-2, and ZO-3. Adherens junctions (AJs), located more basally than TJs, include a series of cadherin proteins of which E-cadherin are highly expressed. Desmosomes and gap junctions seal the intercellular space at the most basolateral side of the cells. Environmental factors may disrupt cell-to-cell adhesion causing a "barrier dysfunction"

(Figure 2A). The conjunctival stroma functions as a mechanically stable and elastic matrix for the epithelium. The conjunctiva contains conjunctiva-associated lymphoid tissue (CALT) in the form of organized lymphoid follicles and subepithelial lymphoid tissue. The CALT together with the lymphoid tissue present in the lacrimal gland and the efferent tear duct system takes part in the antimicrobial defense of the ocular surface.[22–24]

The corneal epithelium is a 5–6 cell layer thick, flat and transparent, non-keratinized stratified squamous epithelium lacking goblet cells, anchored to the Bowman's layer, overlying the avascular corneal stroma (Figure 2B). The basal epithelium at the limbus contains the niche of limbal stem/progenitor cells (LSCs) interacting with a highly vascularized and innervated stroma, stromal cells and extracellular matrix. The limbus is not just the barrier between corneal and conjunctival epithelia but also responsible for maintaining the integrity of the corneal surface and continuous renewal of the corneal epithelium.[25,26]

Cells at the apical layers of both conjunctival and corneal epithelia are sealed by TJs, AJs and desmosomes and express claudins, the most important components of TJ complexes. Conjunctival epithelium expresses claudin-1, -2, -4, -7, -9, -10, and -14, whereas corneal epithelial cells express claudin-1, -2, -3, -4, -7, -9, and -14.[21] Expression of other TJ components such as occludin and ZOs were also detected in corneal and conjunctival epithelial cells.[21] AJs and desmosomes are present throughout the different layers, while hemidesmosomes in the basal layers provide structural integrity and anchoring support by connecting the cytoskeletons of adjoining cells to the underlying basal membrane.[27]

3 | OCULAR ALLERGY

The term allergic conjunctivitis or OA refers to a collection of hypersensitivity disorders affecting primarily the lid and conjunctiva and includes different phenotypes varying from seasonal (SAC) and perennial allergic conjunctivitis (PAC), to the chronic vernal (VKC) and atopic keratoconjunctivitis (AKC), and contact blepharoconjunctivitis.[28]

Successful management of OA involves preventive, non-pharmacologic as well as pharmacologic measures.[29] Over the years various therapeutic options have been developed to achieve symptom control in OA including topical antihistamines, mast cell stabilizers, and dual acting agents with use of corticosteroids and immunomodulators in severe cases. However, in the chronic forms, none of these completely control the signs and symptoms or prevent the recurrences.[29] Ocular surface inflammation in VKC and AKC often leads to severe suffering and complications such as corneal ulcers, tissue remodeling, scars, and rarely LSC deficiency,

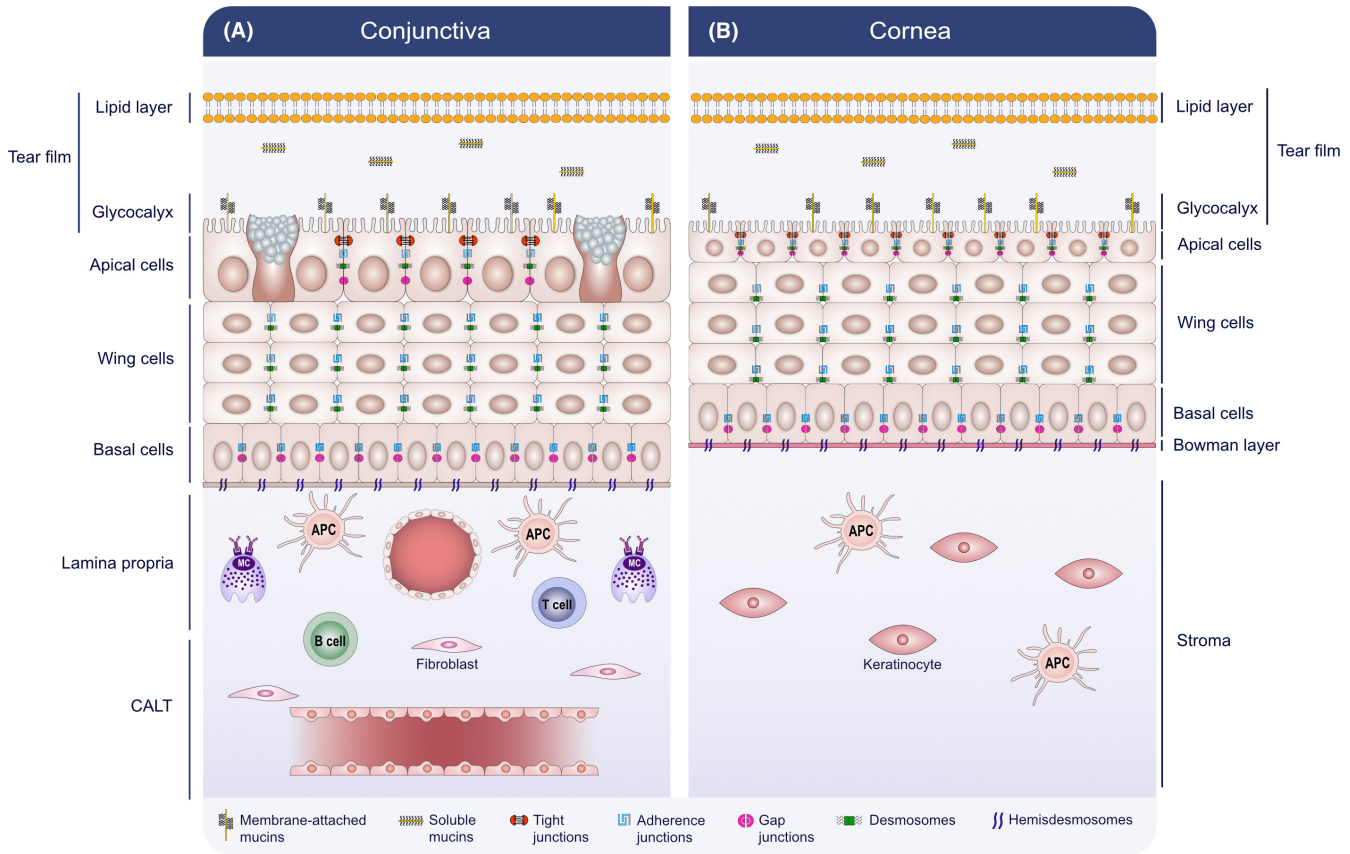

FIGURE 2 Schematic illustration of conjunctival and corneal structure. (A) The conjunctiva comprises of a superficial epithelial layer and a highly vascularized connective tissue containing accessory lacrimal glands, mast cells (MC), the components of the conjunctival associated lymphatic tissue (CALT) T- and B-lymphocytes and antigen-presenting cells (APC) cells within the matrix. (B) The corneal epithelium is a 5–6 cell layer thick, flat and transparent, stratified squamous epithelium lacking goblet cells, covering the avascular corneal stroma made up of an orderly, tightly packed collagen network which ensures transparency, and contain keratocytes and few APC. Apical cells of the stratified epithelium of both corneal and conjunctival epithelium express membrane-associated mucins, which forms the thick glycocalyx at the epithelium-tear film interface. Apical cells are sealed through tight junctions, adherens junctions and desmosomes while hemidesmosomes in the basal layers provide structural integrity and anchoring support by connecting the cytoskeletons of adjoining cells to the underlying substrate. Gap junctions are present at the basal layers. Please note that the proportions of the different components and the different tear film and tissue layers and are not respectful of the anatomical picture

characterized by invasion of conjunctival epithelium into the cornea.[30,31] Further, treatment related complications such as increased intraocular pressure, glaucoma, and cataract arise due to side effects of steroids overuse warranting new and safer therapeutics.[28,32,33] Since the eyes are exposed directly to an outer environment, defective barrier must be primarily involved in the pathology of OA. Epithelial barrier integrity is known to be compromised in dry eye pathologies suggesting that barrier dysfunction plays a critical role in different ocular surface disorders.

3.1 | The ocular surface barrier in OA

In healthy conjunctiva and cornea, TJs constitutes a nearly impermeable barrier, allowing the passage of only nutrients, small size molecules and water, while blocking the passage of pathogens, pollutants, and allergens. Inflammation is a well-known disruptor of the epithelial barrier and is recognized as a cause of several ocular surface diseases, including OA.[34] There is convincing evidence on the altered function and organization of the TJs and abnormal expression of junctional proteins in allergic conjunctivitis with a compromised barrier of the ocular epithelium.[35] Apart from acting as mechanical barrier, conjunctival epithelial cells actively participate in OA contributing to mounting allergic inflammation by expressing and producing cytokines, chemokines, adhesion molecules, and factors that maintain local inflammation leading to tissue remodeling. Conjunctival epithelial cells also play a crucial role in allergic sensitization by instructing type 2 innate lymphoid cells (ILC2) and dendritic cells via release of pro-type 2 cytokines, particularly thymic stromal lymphopoietin (TSLP), IL-33, and IL-23.[36] Interestingly, the papain-induced conjunctival inflammation characterized by eosinophil infiltration and Th2 cytokine overexpression, is IL-33, TSLP, basophils, and ILC2 dependent, highlighting the role of innate immunity in

OA.[37] Further, an animal model of allergic conjunctivitis demonstrated that pollen/TLR4 innate immunity signaling initiates IL-33/ST2 allergic pathway. This triggers a Th2-dominant inflammation suggesting that allergic conjunctivitis is a mucosal epithelial disorder and innate immunity is equally capable of responding to allergens and not just microbes.[38] In another model of OA, uptake and presentation of soluble antigen takes place in CALT with an increased number of antigen-presenting cells and the development of a massive B-cell zone.[24] These mechanisms may partially explain the concept of local allergic conjunctivitis, where conjunctiva act as a uniquely sensitized target organ in allergic patients.[28,39]

3.2 | The role of mucins/glycocalyx in OA

Altered mucin expression has been reported in OA. In OA mouse model, the repeated application of cat dander or peptide P3-1 showed reduced number of goblet cells, with decreased MUC5AC and MUC4 mRNA expression. The goblet cell number and mucin expression levels returned to normal after 24–48 h suggesting a rapid recovery of the protective role of the mucin secretory system.[40] In a goblet cell culture study, IL-4 and IL-13 stimulated conjunctival goblet cell proliferation and mucin secretion[41] indicating the role of Th2-type cytokines in mucus hypersecretion, typical of OA. Clinically, VKC patients showed increased numbers of conjunctival goblet cells with increased expression of MUC5AC, suggesting that a defense mechanism is involved in clearing of allergens from the ocular surface in persistent inflammation.[42] On the contrary, AKC patients have decreased levels of the goblet cell-specific mucin MUC5AC, associated with loss of lubrication and epithelial damage. These patients showed an increased expression of MUC1, MUC2, MUC4, and MUC16 as a defense mechanism to compensate for the loss of protection offered by MUC5AC.[43,44] Thus, the role of mucin barrier appears to be different in different OA phenotypes.

Another possibility is that allergic inflammation alters the glycosylation of transmembrane mucins and impair their affinity to galectin 3, decreasing the barrier function of the glycocalyx. This hypothesis, however, remains to be verified. It is believed that carbohydrate-binding proteins recognize glycan antigens on allergens contributing to a cascade of immune responses. Furthermore, epithelial galectin-3 might regulate inflammatory activities in the allergic response by binding to IgE.[45] Glycosylation is the most complex post-translational modification of proteins, affecting expression and function of numerous proteins required for normal immune function. Using MALDI-TOF MS and MALDI-TOF MS/MS techniques, distinct and unique N-glycome profiles were identified in tears from normal subjects, VKC and AKC.[46] Interestingly, tear proteomic profiles of VKC and AKC showed peaks with increased intensities corresponding to serotransferrin glycans, whereas, peaks with decreased intensity correspond to that of IgA and IgG. This study suggests that allergic inflammation possibly alters N-glycome profile, where glycosylation might play a significant role in ocular surface homeostasis.

3.3 | The epithelial barrier dysfunction in OA

Unlike other allergies, very few studies investigated the role of epithelial barrier dysfunction in OA. An overview of studies suggesting evidence of impaired epithelial barrier functions in OA is presented in Table 1. Ocular surface barrier function was impaired in atopic dermatitis patients with blepharoconjunctivitis. In these patients, the fluorescein uptake by the cornea and conjunctiva was significantly higher than in SAC, PAC, and healthy eyes[47] suggesting that increased epithelial permeability is a consequence of epithelial cell junction disruption. Epithelial barrier integrity is largely maintained by epithelial cell adhesion proteins, such as E-cadherin, CD44 and keratins, the change in expression and function of which leads to a barrier dysfunction. In one of the studies, although the conjunctival epithelium appeared microscopically normal in SAC "out of season" patients as compared to controls, the expression of E-cadherin, CD44 and keratin-14 was significantly reduced indicating a structurally "weaker" epithelium in allergic patients with an increased susceptibility to allergen penetration.[35]

Over the past few years, *in vivo* confocal microscopy (IVCM) has emerged as a powerful tool to detect corneal and conjunctival microstructural changes in patients affected by different ocular surface diseases including OA. In a recent study in VKC, IVCM highlighted inflammation associated corneal changes at the level of epithelium, sub-basal nerve plexus and stroma.[48] These superficial and basal epithelial changes, which are more evident during the inflammatory phases of the disease, may increase the epithelial permeability. Thus, IVCM can be used to study the epithelial barrier dysfunction in OA.

In a mice model of OA, the conjunctival allergen challenge induced a strong immune response with a significant loss in ZO-1 and E-cadherin expression, and the treatment with antihistamine alcaftadine protected this loss associated with eosinophilic allergic inflammation.[49] Further, in patients with history of dry eye and OA, impaired ocular surface was directly implicated in susceptibility to clinical reactions to allergens. Interestingly, when the corneal epithelium was compromised by a controlled adverse environment challenge, patients not only experienced severe symptoms but also showed a heightened allergic response after specific allergen challenge suggesting that patients with both dry eye and OA might have an exaggerated response when exposed to environmental factors.[50] OA and dry eye often coexist, suggesting role of the barrier function in both diseases.[51,52]

A mechanism for barrier dysfunction has been proposed in a mice model of OA, where IL-33/ST2/IL-9/IL-9R signaling pathway exacerbates the allergic inflammation by reducing the expression of TJ proteins. The epithelial-derived IL-33 stimulates the production of IL-9 in CD4+ T cells through ST2 receptor. IL-9 further impairs the ocular surface barrier integrity by binding with IL-9R expressed on the corneal and conjunctival epithelial cells by reducing the expression of

TABLE 1 Overview of studies with evidence of role of barrier epithelium in ocular allergy

Title of the study	Finding	Reference
Tight junction transmembrane protein claudin subtype expression and distribution in human corneal and conjunctival epithelium.	Claudin-1, -4, and -7 expression was observed in both corneal and conjunctival epithelia whereas claudin-10 was predominant in conjunctival epithelium.	21
Reduced structural proteins in the conjunctival epithelium in allergic eye disease.	The expression of epithelial cell adhesion and cytoskeletal proteins (E-cadherins and CD 44) were reduced in out of season SAC. Reduced keratin-14 expression in 'out of season' SAC indicated the possibility of disrupted stratified epithelium.	35
Impression cytology of the conjunctival epithelium in patients with vernal conjunctivitis.	Impression cytology data in VKC patients showed significant alterations in the degree of keratinization and distribution of goblet cells.	42
Impairment of ocular surface epithelium barrier function in patients with atopic dermatitis.	Fluorescein uptake by the cornea and conjunctiva of atopic blepharoconjunctivitis was higher than in SAC, PAC and healthy eyes.	47
Alterations of MUC 1, 2 and 4 tear function and the ocular surface disorder in patients with atopic keratoconjunctivitis.	As compared to healthy eyes, MUC 1, 2 and 4 mRNA expression was found to be considerably higher in eyes with significant epithelial disease.	44
Alterations of the ocular surface epithelial MUC16 and goblet cell MUC5AC in patients with atopic keratoconjunctivitis.	MUC16 mRNA expression was significantly upregulated with substantial downregulation of MUC5AC mRNA expression in eyes with AKC as compared to eyes of control subjects.	43
Comparison of effects of alcaftadine and olopatadine on conjunctival epithelium and eosinophil recruitment in a murine model of allergic conjunctivitis.	Conjunctival allergen challenge induced a significant loss of ZO-1 and E-cadherin expression in challenged and vehicle-treated control groups.	49
Increased conjunctival expression of protease-activated receptor 2 (PAR-2) in seasonal allergic conjunctivitis: a role for abnormal conjunctival epithelial permeability in disease pathogenesis?	Increased expression of conjunctival epithelial PAR-2 in SAC suggests that conjunctival epithelial barrier disruption plays an important role in pathogenesis of allergic conjunctivitis.	97
Localization and expression of zonula occludins-1 in the rabbit corneal epithelium following exposure to benzalkonium chloride.	Preservatives in eye drops disrupts the tight junction proteins ZO-1 and ZO-2 in rabbit corneal epithelium.	113
Exacerbation of signs and symptoms of allergic conjunctivitis by a controlled adverse environment challenge in subjects with a history of dry eye and ocular allergy.	Exposure to allergens disrupts ocular surface barrier which further exacerbates clinical reactions to these allergens.	50
IL-33/ST2/IL-9/IL-9R signaling disrupts ocular surface barrier in allergic inflammation.	IL-33/ST2/IL-9/IL-9R signaling pathway is involved in corneal and conjunctival epithelium barrier disruption in allergic conjunctivitis.	53
Conjunctival transcriptome analysis reveals the overexpression of multiple pattern recognition receptors (PRR) in VKC.	Increased expression of multiple PRR related genes, genes encoding pro-inflammatory cytokines (IL-6, CCL24, CCL18, CXCL1, ICAM-1, TGFβ1) were observed in more severe form of VKC.	54

Abbreviations: AKC, atopic keratoconjunctivitis; MUC, mucin; SAC, seasonal allergic conjunctivitis; VKC, vernal keratoconjunctivitis.

ZO-1, claudin-1, occludin and E-cadherin.[53] In a recent transcriptomic study in VKC, conjunctival epithelial cells showed an increased expression of the pro-inflammatory genes (IL-6, CCL24, CCL18, CXCL1, ICAM-1, TGFβ-1), which correlated with increased disease severity score and corneal staining, reflecting the epithelial dysfunction in VKC.[54] The events associated with ocular epithelial barrier dysfunction occurring in different forms of OA are summarized in Table 2.

3.4 | Alteration in epithelial barrier repair in OA

In response to injury, epithelial cells lose cell attachments, begin to divide, migrate rapidly to the injury site, and may lose the expression of TJ proteins ZO-1, occludin, E-cadherin and other markers of mature differentiated epithelium. This process is known as epithelial to mesenchymal transition (EMT), a phenomenon where epithelial cells take on a migratory mesenchymal phenotype as a normal tissue repair through multiple inflammatory pathways.[55] Mesenchymal cells derived from the basal epithelium begin to produce vimentin, desmin, fibronectin, tenascin, laminin, collagens and other proteins, which forms a makeshift matrix to protect and cover the exposed basement membrane and start expressing differentiation markers, such as alpha-smooth muscle actin. Studies have shown that type 2 inflammatory diseases, such as asthma[56] and AR,[17] often exhibit a chronic EMT-based ongoing injury-repair cycle. EMT dysregulation is involved in asthma remodeling,[57,58] but has also been recognized in ocular graft versus host disease.[59]

TABLE 2 Barrier dysfunction in different ocular allergic diseases

Ocular allergic disease	Events associated with ocular epithelial barrier dysfunction
SAC/PAC	• Altered expression of epithelial cell adhesion proteins • Increased protease receptors • Increased epithelial permeability • Tear film dysfunctions
VKC	• Possible altered expression of epithelial cell adhesion proteins • Increased epithelial permeability • Increased number of goblet cells • Increased secretion of conjunctival derived mucin • Increased expression of pathogen pattern receptors • Increased expression of proteases • Altered N-glycome profile • Altered conjunctival microbiota
AKC	• Possible altered expression of epithelial cell adhesion proteins • Increased epithelial permeability • Decreased number of goblet cells • Decreased secretion of conjunctival derived mucin • Increased compensatory secretion of mucins • Altered N-glycome profile • Overlaps with dry eye disease

Abbreviations: AKC, atopic keratoconjunctivitis; MUC, mucin; PAC, perennial allergic conjunctivitis; SAC, seasonal allergic conjunctivitis; VKC, vernal keratoconjunctivitis.

In the respiratory tract, the epithelial/mesenchymal interface, also known as epithelial-mesenchymal trophic unit (EMTU), plays a major role in development, repair and homeostasis.[60] In the eye, EMTU dysregulation and EMT may lead to completely different phenotypes from the upper tarsal giant papillae formation typical of VKC[61] to the progressive shortening and subepithelial fibrosis of mucous membrane pemphigoid or other cicatrizing conjunctivitis such as severe AKC. There is also growing evidence that autophagy modulates remodeling in airways[62] and possibly in VKC, where autophagy markers, LC3B, Cathepsin D, Beclin-1 and LAMP1 were significantly upregulated in conjunctival inflamed VKC tissues.[63] The enhanced autophagy in conjunctival fibroblast cultures suggests that modulators of autophagy could be a new therapeutic strategy in VKC.[63] We suggest that the epithelial barrier and EMTU are critical components facilitating allergens/pollutants absorption, sensitization, and the vicious circle of OA (Figure 3).

4 | FACTORS CONTRIBUTING TO OCULAR BARRIER DYSREGULATION IN OA

4.1 | The exposome

The exposome is defined as the measure of all environmental exposures of an individual in a lifetime and how these exposures affect the health.[64,65] A general external environment (urban environment, climate factors, social capital, stress, etc.), a specific external environment (diet, physical activity, tobacco, infections, etc.), and an internal environment (metabolic factors, gut microflora, inflammation, oxidative stress, etc.) complementing the genome, are the main causes of the chronic diseases including allergy and probably most ocular surface diseases (Figure 4).

4.2 | Genetic and epigenetic factors

Several allergic susceptibility candidate genes are known to regulate epithelial barrier homeostasis. Studies have identified these risk susceptibility genes associated with epithelial barrier dysfunction in AD, asthma, and eosinophilic esophagitis. Null gene mutations of filaggrin (FLG) are the major risk factors for AD and eosinophilic esophagitis. Polymorphism in asthma susceptibility genes (Protocadherin-1 (PCDH1), 80 cadherin-related family member 3 (CDHR3), serine peptidase inhibitor, Kazal type 5 (SPINK5), and orosomucoid-like 3 (ORMDL3)) is significantly associated with aberrant epithelial barrier.[66] In asthma, the genes relevant to epithelial barrier function (*ZMYND10*, *CDH26*, *CDHR3*) have been shown to be differentially methylated.[67] Identification of risk susceptibility genes in VKC with respect to ocular epithelial barrier will help to better understand the disease pathogenesis. The epigenetic mechanisms of epithelial barrier dysfunction and remodeling is an emerging area of investigation that should be explored in OA.

4.3 | Role of hormones in epithelial barrier function

The role of hormones is crucial in maintaining epithelial barrier homeostasis and integrity as evident from studies on deficiencies in wound healing and epithelial dysfunction in patients with thyroid disease and diabetes.[68] Sex hormones, insulin, IGF-1 and glucocorticoids are

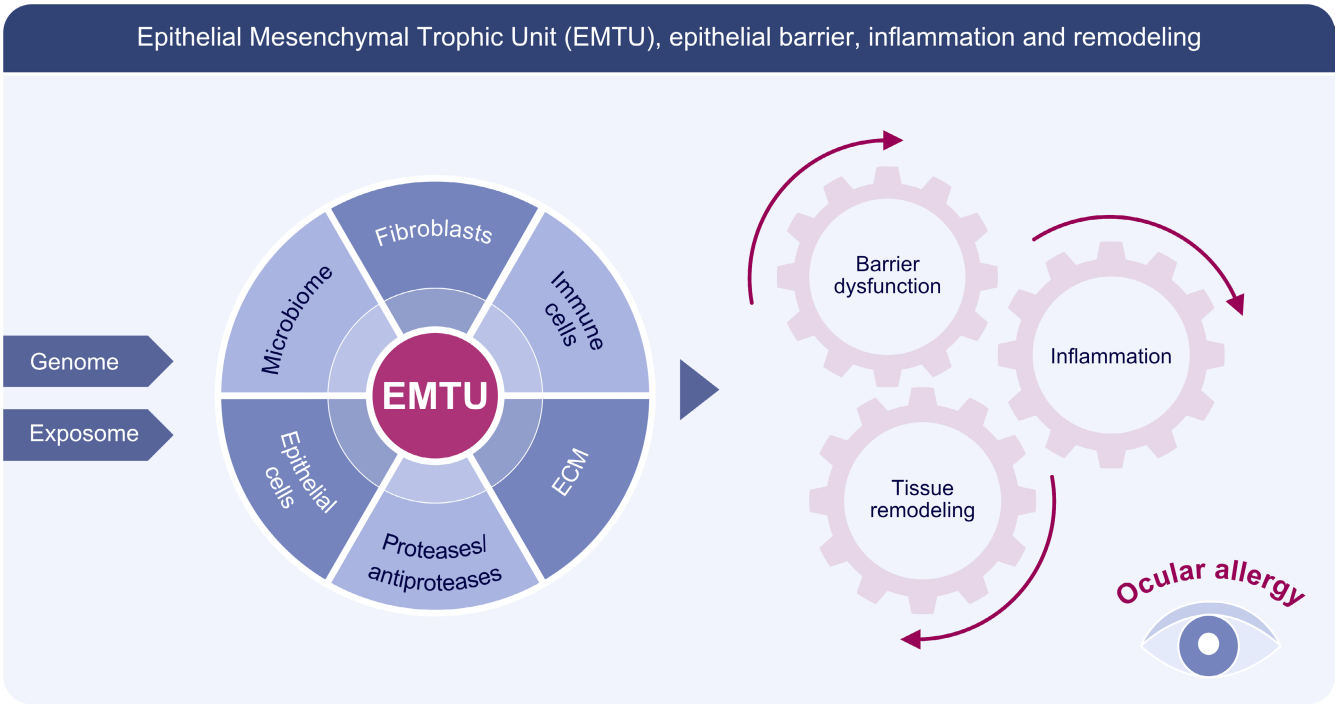

FIGURE 3 The role of the epithelial barrier and epithelial-mesenchymal trophic unit (EMTU) as a central player in development, repair and homeostasis of the ocular surface. Reciprocal interactions and modulation between epithelial cells, fibroblasts, immune cells, extracellular matrix (ECM), protease-antiprotease imbalance contribute to the EMTU function. Dysregulation of EMTU favored by genetic factors and exposome may lead to barrier dysfunction, inflammation and tissue remodeling and the vicious circle of ocular allergy

known to regulate epithelial cell differentiation. However, the hormonal regulation of disruption of epithelial homeostasis and allergic response is poorly understood. Studies have reported the link between insulin resistance in children and adults with asthma, as well as positive association of asthma and AD with pre-diabetes.[69] Significant changes were observed in the serum hormonal profiles of prepubertal non-obese allergic children and in chronic rhinosinusitis patients.[70] In OA, a hormonal dysfunction has been suggested in VKC, which affects usually (but not only) boys in prepubertal age. Furthermore, different circulating sex and growth hormone levels have been reported in different phases of VKC.[71] The exact mechanisms of VKC resolution after puberty in the majority of patients remains unknown.

4.4 | Early-life events, microbiome and epithelial barrier disruption

Any disturbance in epithelial barrier morphogenesis and developmental processes in early childhood could significantly impact the function of adult epithelium. Evidence suggests the role of epithelial structure remodeling in development of childhood asthma. Altered Wnt,[72] Hippo, Notch/Jagged,[73] and Hedgehog developmental pathways[74] significantly correlated with epithelial remodeling representing a crucial event in initiation and progression of allergy. Mouse models of the atopic march demonstrated that sensitization to allergens through disrupted skin barrier is sufficient to elicit an immune response at other barrier sites.[75] Early-life alteration in gut microbiome composition could be responsible for aberrant epithelial responses with a predisposition to allergic sensitization.[76] It is still not clear if an early exposure to specific factors could modify the ocular surface barrier.

Accumulating evidence suggests that there is a possible link between microbiota dysbiosis, epithelial barrier disruption and development of allergies.[77] Intestinal microbiota not only maintains a symbiotic relationship with the host but prevents pathogenic bacteria from accessing the epithelial barrier and promotes a healthy barrier. Bacterial dysbiosis is rather considered as a hallmark of AD,[78] possibly in asthma[79] and in food allergy.[80] Microbiota composition might significantly influence intestinal epithelial barrier function by altering TJ protein composition and the expression of ZO-1, ZO-2 and occludin.[81] The ocular surface is characterized by a relatively stable, low diversity, "minimal" core microbiome where all individuals share a few taxa.[82] Culture-independent methods such as 16S rRNA gene sequencing have shown that *Protoebacteria*, *Firmicutes* and *Actinobacteria* are the most abundant phyla on the ocular surface. At the genera-level, studies have consistently reported higher prevalence of *Corynebacterium*, *Staphylococcus*, *Propionibacterium*, *Streptococcus*, *Acinetobacter* and *Pseudomonas*.[82,83] There is an increasing interest in the role of the conjunctival microbiome in different ocular diseases, such as meibomian gland dysfunction,[84] dry eye,[85,86] contact lens wear,[87] Steven-Johnson Syndrome,[88] bacterial and fungal keratitis.[89] The role of altered gut microbiota in the development of uveitis[90] and dry eye[91] has been also established. Interestingly, the multiple

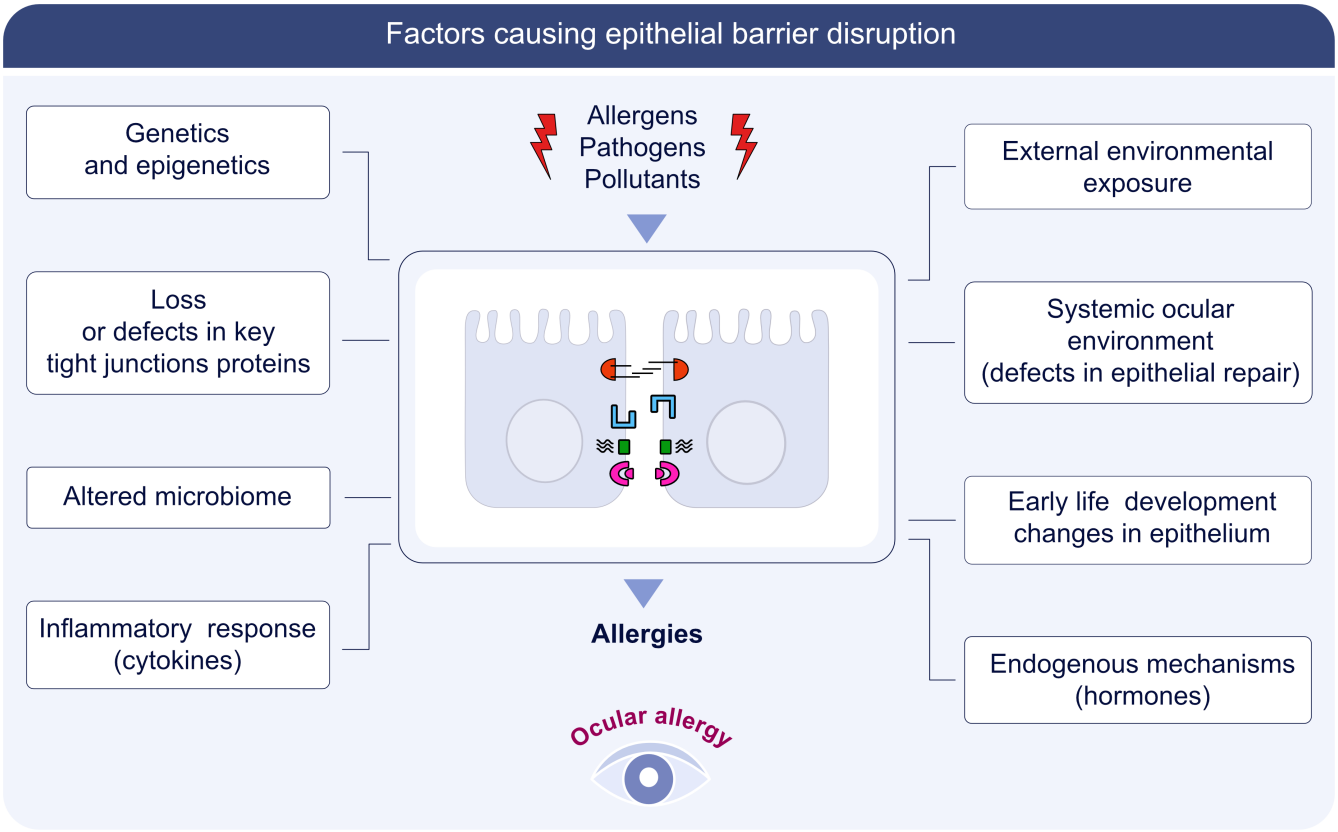

FIGURE 4 Factors affecting epithelial barrier function. Barrier dysregulation can be induced by loss or defects in major TJs and adhesion proteins, disruption of barrier by exposome (proteases, pollutants, chemical injury, trauma), inflammatory responses (barrier disrupting Th2 cytokines) and endogenous mechanisms such as altered metabolism, microbiome or imbalance of hormones regulating epithelial homeostasis

expression of PRRs in VKC suggests a role of host-pathogens interaction in the development of VKC.[54] Recently, bacterial and fungal dysbiosis has been described in VKC, suggesting that altered conjunctival microbiota composition might be involved in the disease pathogenesis.[92] In VKC, *Bacteroidetes* and *Fusobacteria* constitute the core bacterial microbiome, which includes different species of gram-negative bacteria, whereas the fungal microbiome analysis revealed increased abundance of *Malasseziaceae* family members.[91] In another study, SAC/PAC patients exhibited a significantly different microbiome as compared to VKC, with *Brevibacterium auranticum* and *Staphylococcus sciuri* species more predominant in SAC/PAC and *Streptococcus* species in VKC.[93]

4.5 | Allergens and protease mediated barrier dysregulation

Allergens with intrinsic proteolytic activity can cross the conjunctival epithelium to enter the submucosal space where they may interact with dendritic cells and conjunctival mast cells to induce allergic inflammation. The mechanism by which allergens cross the epithelial barrier is by degradation of epithelial TJs and E-cadherin destabilization via their proteolytic activity. The loss of epithelial barrier integrity could also occur via activation of protease-activated receptors 2 (PAR-2), highly expressed on epithelial cells.[94] The PAR-2 activation leads to epithelial degradation by activating inflammatory signaling pathways.[95] Pollen proteases degrade epithelial TJs by direct cleavage of the extracellular domains of occludin, claudin-1, and ZO-1.[18,96]

Interestingly, conjunctival PAR-2 was significantly upregulated in SAC confirming the epithelial disruption related to allergen exposure.[97] Similarly in a murine model of OA, allergen challenge caused a significant decrease in expression of junctional proteins ZO-1, suggesting that allergens compromise the epithelial barrier function.[49] Several proteases, such as tryptase, chymase, urokinase-type, tissue type plasminogen activators, and metalloproteases were found to be overexpressed in tears and tissues of patients affected by VKC.[98,99] Therefore, a dysregulated proteases/antiproteases balance might play a key role in the barrier dysregulation in OA.

4.6 | Effect of pollution on the ocular surface

People living in urban areas are exposed to higher level of air pollution experiencing discomfort such as ocular itching, redness, burning, foreign body sensation and reduced tear production.[100] Studies have shown that air pollutants aggravate the signs and symptoms of dry eye and OA and possibly alter the barrier function.[101,102] Further,

an impaired epithelial barrier could expose deeper tissues to air pollutants leading to activation of immune cells. Studies in experimental models demonstrated that exposure to PM leads to a significant thickening of corneal and conjunctival epithelial layers.[103] If these changes are prolonged over time, they may irreversibly change the refractive power of the cornea and the vision process.[104] Corneal epithelial cells exposed to different concentrations of PM particles release pro-inflammatory cytokines, IL-6, IL-8, TNF-α, IL-1β, and MCP-1,[105] and show decreased cellular viability and proliferation with altered mucin production.[106] Interestingly, conjunctival epithelial cells can easily trap diesel exhaust particles (DEP), leading to oxidative stress in epithelial tissues followed by an IL-6-mediated inflammatory response.[107] The resulting antioxidant response and the increased mucin expression by conjunctival epithelial cells can be considered an adaptive response to oxidative stress triggered by DEP. Similarly, cigarette smoke extract disrupts the structural integrity of the superficial corneal epithelium damaging the intact epithelial barrier with enhanced oxidative stress response at the same time.[108] The role of oxidative stress has been established in several ocular surface diseases such as dry eyes, uveitis, allergic keratoconjunctivitis.[109] Interestingly, higher levels of hydrogen peroxide, a marker of oxidative stress were observed in serum of SAC[110] and in serum and tears of active phase of VKC.[111] Furthermore, in a recent study in mice model of OA, repeated topical applications of a mixture of particulate air pollutants and pollen extract induced eosinophilic conjunctivitis suggesting that particulate pollutants probably act as adjuvants in development of allergic conjunctivitis.[112] How pollutants aggravate OA and whether conjunctival epithelial integrity is altered by pollution and/or oxidative stress, is an area of further investigation.

5 | OCULAR EPITHELIAL BARRIER AS THERAPEUTIC TARGET

The treatment of OA includes the use of topical antihistamines, mast cell stabilizers, dual acting agents and corticosteroids and immunomodulators in severe cases.[29] The current therapies are not completely effective in controlling signs and symptoms or preventing recurrences. Most ophthalmic preparations contain preservatives such as benzalkonium chloride, which is known to disrupt TJs of corneal epithelium.[113] Tissue specific barrier restoration therapies are found to be effective in asthma, rhinitis and dermatitis. It is well known that OA flares up on exposure of environment nonspecific factors and pollutants. Therefore, restoration of the barrier function of the ocular surface could be an alternative therapy for effective management of OA. Identifying key processes/mediators involved in restoration of the homeostatic state of the epithelial barrier is vital to develop novel therapeutic approaches for OA. Corticosteroids restores epithelial barrier function by suppressing the inflammatory response but how they preserve barrier integrity is not clear. In addition, the prolonged use of corticosteroids should be avoided because of the serious ocular side effects such as cataract and glaucoma.

BOX 2 Future research perspectives

- Characterization of the conjunctival epithelial junctional complexes and specific endotypes in different ocular allergic phenotypes should be investigated.
- Interaction between local (conjunctival) and gut microbiota and its correlation with the ocular barrier function in physiological conditions and different ocular allergic diseases need to be evaluated.
- The link between epithelial dysfunction and tissue remodeling is partially known. The concepts of EMTU and EMT remain to be explored in OA.
- Further research in genetic and epigenetics in OA is required.
- Interaction between hormones and development of OA especially in VKC need to be studied.
- Developing new topical barrier formulations for OA will not only protect the ocular surface but will also help to restore a disrupted epithelial barrier in patients affected by severe chronic allergies such as VKC and AKC.

The non-pharmacological strategies to prevent allergy include the avoidance of pollutants and allergens, protection of normal microbiota, Vitamin D supplementation, use of barrier creams in AD and mechanical gels in AR. Therapies targeting epithelial barrier defects can be promising strategies for preventing allergic diseases in future. Studies on restoration of epithelial barrier *in vitro* and *in vivo* in mouse models have shown promising results.[66] Barrier gel formulation have been developed for rhinitis and dermatitis. For example, the barrier repair agent calycosin alleviated allergic contact dermatitis by repairing epithelial TJ via down-regulating HIF-1α, suggesting that HIF- 1α and TJs could be potential therapeutic targets for allergic dermatitis.[114] The effect of another allergen-blocker mechanical barrier gel was shown to improve symptoms and quality of life in AR patients.[115] A topical barrier formulation containing chitosan-glycerol was found effective in preventing the absorption of nickel ions into the skin potentially reducing the chances of metal allergy.[116] Considering the structural conjunctival changes in OA, developing novel formulations that preserves and restore ocular barrier integrity is the pressing need.

6 | CONCLUSIONS

Whether barrier dysfunction precedes and predisposes to OA development is still not clearly understood; however, it maintains and contributes to the vicious cycle of allergic inflammation by facilitating paracellular transport of allergens, pathogens, pollutants and other harmful triggers. Further studies on how barrier disruption affects allergic sensitization and chronicity of inflammation especially in VKC and AKC would offer scope of better management of these

conditions. Future research should focus on developing therapies that preserves epithelial barrier integrity as the physical barrier formulations can help in prevention of different types of OA (Box 2).

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CONFLICT OF INTEREST

The author declares that they have no competing interests.

METHOD OF LITERATURE SEARCH

The literature searched was performed using the defined key words such as epithelial barrier, barrier dysfunction, allergy and epithelial barrier, ocular epithelium and factors affecting epithelial barrier. PubMed and Google scholar were accessed for relevant reviews and primary articles on allergy and barrier dysfunction.

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REFERENCES

1. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? *Nat Rev Immunol*. 2021;21(11):739-751.
2. Schleimer RP, Berdnikovs S. Etiology of epithelial barrier dysfunction in patients with type 2 inflammatory diseases. *J Allergy Clin Immunol*. 2017;139:1752-1761.
3. Paulsen F. Functional anatomy and immunological interactions of ocular surface and adnexa. *Dev Ophthalmol*. 2008;41:21-35.
4. Torricelli AA, Singh V, Santhiago MR, Wilson SE. The corneal epithelial basement membrane: structure, function, and disease. *Invest Ophthalmol Vis Sci*. 2013;54:6390-6400.
5. Martinez-Carrasco R, Argueso P, Fini ME. Membrane-associated mucins of the human ocular surface in health and disease. *Ocul Surf*. 2021;21:313-330.
6. Blalock TD, Spurr-Michaud SJ, Tisdale AS, et al. Functions of MUC16 in corneal epithelial cells. *Invest Ophthalmol Vis Sci*. 2007;48:4509-4518.
7. Mantelli F, Argueso P. Functions of ocular surface mucins in health and disease. *Curr Opin Allergy Clin Immunol*. 2008;8:477-483.
8. Gipson IK. Distribution of mucins at the ocular surface. *Exp Eye Res*. 2004;78:379-388.
9. Argueso P, Guzman-Aranguez A, Mantelli F, Cao Z, Ricciuto J, Panjwani N. Association of cell surface mucins with galectin-3 contributes to the ocular surface epithelial barrier. *J Biol Chem*. 2009;284:23037-23045.
10. Dhar P, McAuley J. The role of the cell surface mucin MUC1 as a barrier to infection and regulator of inflammation. *Front Cell Infect Microbiol*. 2019;9:117.
11. Menon BB, Kaiser-Marko C, Spurr-Michaud S, Tisdale AS, Gipson IK. Suppression of Toll-like receptor-mediated innate immune responses at the ocular surface by the membrane-associated mucins MUC1 and MUC16. *Mucosal Immunol*. 2015;8:1000-1008.
12. Dhar P, Sarkar S, Ng GZ, et al. Effect of MUC1 length polymorphisms on the NLRP3 inflammasome response of human macrophages. *Hum Immunol*. 2019;80:878-882.
13. Sugita K, Kabashima K. Tight junctions in the development of asthma, chronic rhinosinusitis, atopic dermatitis, eosinophilic esophagitis, and inflammatory bowel diseases. *J Leukoc Biol*. 2020;107:749-762.
14. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol*. 2014;134:509-520.
15. Xiao C, Puddicombe SM, Field S, et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol*. 2011;128:549-556 e1-12.
16. Yuki T, Tobiishi M, Kusaka-Kikushima A, Ota Y, Tokura Y. Impaired tight junctions in atopic dermatitis skin and in a skin-equivalent model treated with interleukin-17. *PLoS One*. 2016;11:e0161759.
17. Steelant B, Farre R, Wawrzyniak P, et al. Impaired barrier function in patients with house dust mite-induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. *J Allergy Clin Immunol*. 2016;137:1043-1053 e5.
18. Runswick S, Mitchell T, Davies P, Robinson C, Garrod DR. Pollen proteolytic enzymes degrade tight junctions. *Respirology*. 2007;12:834-842.
19. Fukuoka A, Matsushita K, Morikawa T, Takano H, Yoshimoto T. Diesel exhaust particles exacerbate allergic rhinitis in mice by disrupting the nasal epithelial barrier. *Clin Exp Allergy*. 2016;46:142-152.
20. Schamberger AC, Mise N, Jia J, et al. Cigarette smoke-induced disruption of bronchial epithelial tight junctions is prevented by transforming growth factor-beta. *Am J Respir Cell Mol Biol*. 2014;50:1040-1052.
21. Yoshida Y, Ban Y, Kinoshita S. Tight junction transmembrane protein claudin subtype expression and distribution in human corneal and conjunctival epithelium. *Invest Ophthalmol Vis Sci*. 2009;50:2103-2108.
22. Knop N, Knop E. Conjunctiva-associated lymphoid tissue in the human eye. *Invest Ophthalmol Vis Sci*. 2000;41:1270-1279.
23. Knop E, Knop N. A functional unit for ocular surface immune defense formed by the lacrimal gland, conjunctivaand lacrimal drainage system. *Adv Exp Med Biol*. 2002;506:835-844.
24. Steven P, Schwab S, Kiesewetter A, Saban DR, Stern ME, Gehlsen U. Disease-specific expression of conjunctiva associated lymphoid tissue (CALT) in mouse models of dry eye disease and ocular allergy. *Int J Mol Sci*. 2020;21:7514.
25. Bonnet C, Gonzalez S, Roberts JS, et al. Human limbal epithelial stem cell regulation, bioengineering and function. *Prog Retin Eye Res*. 2021;85:100956.
26. Schermer A, Galvin S, Sun TT. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. *J Cell Biol*. 1986;103:49-62.
27. Mantelli F, Mauris J, Argueso P. The ocular surface epithelial barrier and other mechanisms of mucosal protection: from allergy to infectious diseases. *Curr Opin Allergy Clin Immunol*. 2013;13:563-568.
28. Leonardi A, Bogacka E, Fauquert JL, et al. Ocular allergy: recognizing and diagnosing hypersensitivity disorders of the ocular surface. *Allergy*. 2012;67:1327-1337.
29. Leonardi A, Silva D, Perez Formigo D, et al. Management of ocular allergy. *Allergy*. 2019;74:1611-1630.
30. Bonnet C, Roberts JS, Deng SX. Limbal stem cell diseases. *Exp Eye Res*. 2021;205:108437.
31. Donthineni PR, Varma S, Kethiri A, et al. Histopathological characteristics of limbal stem cell deficiency secondary to chronic vernal keratoconjunctivitis. *Cornea*. 2021. doi:10.1097/ICO.0000000000002775. Epub ahead of print. PMID: 34116542.
32. Jabbehdari S, Starnes TW, Kurji KH, et al. Management of advanced ocular surface disease in patients with severe atopic keratoconjunctivitis. *Ocul Surf*. 2019;17:303-309.
33. Feizi S, Javadi MA, Alemzadeh-Ansari M, Arabi A, Shahraki T, Kheirkhah A. Management of corneal complications in vernal keratoconjunctivitis: a review. *Ocul Surf*. 2021;19:282-289.

34. Contreras-Ruiz L, Schulze U, Garcia-Posadas L, et al. Structural and functional alteration of corneal epithelial barrier under inflammatory conditions. *Curr Eye Res*. 2012;37:971-981.
35. Hughes JL, Lackie PM, Wilson SJ, Church MK, McGill JI. Reduced structural proteins in the conjunctival epithelium in allergic eye disease. *Allergy*. 2006;61:1268-1274.
36. Xiong Y, Cui X, Li W, et al. BLT1 signaling in epithelial cells mediates allergic sensitization via promotion of IL-33 production. *Allergy*. 2019;74:495-506.
37. Sugita J, Asada Y, Ishida W, et al. Contributions of Interleukin-33 and TSLP in a papain-soaked contact lens-induced mouse conjunctival inflammation model. *Immun Inflamm Dis*. 2017;5:515-525.
38. Li J, Zhang L, Chen X, et al. Pollen/TLR4 Innate Immunity Signaling Initiates IL-33/ST2/Th2 Pathways in Allergic Inflammation. *Sci Rep*. 2016;6:36150.
39. Yamana Y, Fukuda K, Ko R, Uchio E. Local allergic conjunctivitis: a phenotype of allergic conjunctivitis. *Int Ophthalmol*. 2019;39:2539-2544.
40. Kunert KS, Keane-Myers AM, Spurr-Michaud S, Tisdale AS, Gipson IK. Alteration in goblet cell numbers and mucin gene expression in a mouse model of allergic conjunctivitis. *Invest Ophthalmol Vis Sci*. 2001;42:2483-2489.
41. Garcia-Posadas L, Hodges RR, Diebold Y, Dartt DA. Context-dependent regulation of conjunctival goblet cell function by allergic mediators. *Sci Rep*. 2018;8:12162.
42. Aragona P, Romeo GF, Puzzolo D, Micali A, Ferreri G. Impression cytology of the conjunctival epithelium in patients with vernal conjunctivitis. *Eye (Lond)*. 1996;10(Pt 1):82-85.
43. Dogru M, Matsumoto Y, Okada N, et al. Alterations of the ocular surface epithelial MUC16 and goblet cell MUC5AC in patients with atopic keratoconjunctivitis. *Allergy*. 2008;63:1324-1334.
44. Dogru M, Okada N, Asano-Kato N, et al. Alterations of the ocular surface epithelial mucins 1, 2, 4 and the tear functions in patients with atopic keratoconjunctivitis. *Clin Exp Allergy*. 2006;36:1556-1565.
45. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol*. 2008;8:205-217.
46. Messina A, Palmigiano A, Tosto C, et al. Tear N-glycomics in vernal and atopic keratoconjunctivitis. *Allergy*. 2021;76:2500-2509.
47. Yokoi K, Yokoi N, Kinoshita S. Impairment of ocular surface epithelium barrier function in patients with atopic dermatitis. *Br J Ophthalmol*. 1998;82:797-800.
48. Modugno RL, Scalora T, Bonaldo A, Lazzarini D, Leonardi A. Corneal microstructural changes by confocal microscopy in vernal keratoconjunctivitis patients treated with topical cyclosporine. *Ocul Immunol Inflamm*. 2020;1-7. doi:10.1080/09273948.2020.1745243. Epub ahead of print. PMID: 32275180.
49. Ono SJ, Lane K. Comparison of effects of alcaftadine and olopatadine on conjunctival epithelium and eosinophil recruitment in a murine model of allergic conjunctivitis. *Drug Des Devel Ther*. 2011;5:77-84.
50. Gomes PJ, Ousler GW, Welch DL, Smith LM, Coderre J, Abelson MB. Exacerbation of signs and symptoms of allergic conjunctivitis by a controlled adverse environment challenge in subjects with a history of dry eye and ocular allergy. *Clin Ophthalmol*. 2013;7:157-165.
51. Leonardi A, Modugno RL, Salami E. Allergy and dry eye disease. *Ocul Immunol Inflamm*. 2021;5:1-9.
52. Hom MM, Nguyen AL, Bielory L. Allergic conjunctivitis and dry eye syndrome. *Ann Allergy Asthma Immunol*. 2012;108:163-166.
53. Hu J, Gao N, Zhang Y, et al. IL-33/ST2/IL-9/IL-9R signaling disrupts ocular surface barrier in allergic inflammation. *Mucosal Immunol*. 2020;13:919-930.
54. Leonardi A, Daull P, Garrigue JS, et al. Conjunctival transcriptome analysis reveals the overexpression of multiple pattern recognition receptors in vernal keratoconjunctivitis. *Ocul Surf*. 2021;19:241-248.
55. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell*. 2009;139:871-890.
56. Hackett TL. Epithelial-mesenchymal transition in the pathophysiology of airway remodelling in asthma. *Curr Opin Allergy Clin Immunol*. 2012;12:53-59.
57. Johnson JR, Roos A, Berg T, Nord M, Fuxe J. Chronic respiratory aeroallergen exposure in mice induces epithelial-mesenchymal transition in the large airways. *PLoS One*. 2011;6:e16175.
58. Fischer KD, Hall SC, Agrawal DK. Vitamin D supplementation reduces induction of epithelial-mesenchymal transition in allergen sensitized and challenged mice. *PLoS One*. 2016;11:e0149180.
59. Ogawa Y, Shimmura S, Kawakita T, Yoshida S, Kawakami Y, Tsubota K. Epithelial mesenchymal transition in human ocular chronic graft-versus-host disease. *Am J Pathol*. 2009;175:2372-2381.
60. Evans MJ, Van Winkle LS, Fanucchi MV, Plopper CG. The attenuated fibroblast sheath of the respiratory tract epithelial-mesenchymal trophic unit. *Am J Respir Cell Mol Biol*. 1999;21:655-657.
61. Leonardi A, Di Stefano A, Motterle L, Zavan B, Abatangelo G, Brun P. Transforming growth factor-beta/Smad - signalling pathway and conjunctival remodelling in vernal keratoconjunctivitis. *Clin Exp Allergy*. 2011;41:52-60.
62. Poon AH, Chouiali F, Tse SM, et al. Genetic and histologic evidence for autophagy in asthma pathogenesis. *J Allergy Clin Immunol*. 2012;129:569-571.
63. Brun P, Tarricone E, Di Stefano A, et al. The regulatory activity of autophagy in conjunctival fibroblasts and its possible role in vernal keratoconjunctivitis. *J Allergy Clin Immunol*. 2020;146:1210-1213 e9.
64. Vrijheid M. The exposome: a new paradigm to study the impact of environment on health. *Thorax*. 2014;69:876-878.
65. Alkotob SS, Cannedy C, Harter K, et al. Advances and novel developments in environmental influences on the development of atopic diseases. *Allergy*. 2020;75:3077-3086.
66. Hellings PW, Steelant B. Epithelial barriers in allergy and asthma. *J Allergy Clin Immunol*. 2020;145:1499-1509.
67. Qi C, Jiang Y, Yang IV, et al. Nasal DNA methylation profiling of asthma and rhinitis. *J Allergy Clin Immunol*. 2020;145:1655-1663.
68. Salazar JJ, Ennis WJ, Koh TJ. Diabetes medications: impact on inflammation and wound healing. *J Diabetes Complications*. 2016;30:746-752.
69. Garmendia JV, Moreno D, Garcia AH, De Sanctis JB. Metabolic syndrome and asthma. *Recent Pat Endocr Metab Immune Drug Discov*. 2014;8:60-66.
70. Berdnikovs S, Abdala-Valencia H, Loffredo LF, et al. Systemic imbalance in hormone levels associates with epithelial barrier dysfunction in allergic disease. *J Allergy Clin Immunol*. 2017;139:1.
71. Sacchetti M, Lambiase A, Moretti C, Mantelli F, Bonini S. Sex hormones in allergic conjunctivitis: altered levels of circulating androgens and estrogens in children and adolescents with vernal keratoconjunctivitis. *J Immunol Res*. 2015;2015:945317.
72. Barreto-Luis A, Corrales A, Acosta-Herrera M, et al. A pathway-based association study reveals variants from Wnt signalling genes contributing to asthma susceptibility. *Clin Exp Allergy*. 2017;47:618-626.
73. Demehri S, Liu Z, Lee J, et al. Notch-deficient skin induces a lethal systemic B-lymphoproliferative disorder by secreting TSLP, a sentinel for epidermal integrity. *PLoS Biol*. 2008;6:e123.
74. Furmanski AL, Saldana JI, Ono M, et al. Tissue-derived hedgehog proteins modulate Th differentiation and disease. *J Immunol*. 2013;190:2641-2649.
75. Bantz SK, Zhu Z, Zheng T. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. *J Clin Cell Immunol*. 2014;5:202.
76. Ruff WE, Greiling TM, Kriegel MA. Host-microbiota interactions in immune-mediated diseases. *Nat Rev Microbiol*. 2020;18:521-538.
77. Yu LC. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. *J Biomed Sci*. 2018;25:79.

78. Meylan P, Lang C, Mermoud S, et al. Skin colonization by staphylococcus aureus precedes the clinical diagnosis of atopic dermatitis in infancy. *J Invest Dermatol*. 2017;137:2497-2504.
79. Huang YJ, Nelson CE, Brodie EL, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol*. 2011;127:372-381 e1-3.
80. Samadi N, Klems M, Untersmayr E. The role of gastrointestinal permeability in food allergy. *Ann Allergy Asthma Immunol*. 2018;121:168-173.
81. Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr*. 2011;141:769-776.
82. Ozkan J, Willcox MD. The ocular microbiome: molecular characterisation of a unique and low microbial environment. *Curr Eye Res*. 2019;44:685-694.
83. Delbeke H, Younas S, Casteels I, Joossens M. Current knowledge on the human eye microbiome: a systematic review of available amplicon and metagenomic sequencing data. *Acta Ophthalmol*. 2021;99:16-25.
84. Dong X, Wang Y, Wang W, Lin P, Huang Y. Composition and diversity of bacterial community on the ocular surface of patients with meibomian gland dysfunction. *Invest Ophthalmol Vis Sci*. 2019;60:4774-4783.
85. de Paiva CS, Jones DB, Stern ME, et al. Altered mucosal microbiome diversity and disease severity in Sjogren syndrome. *Sci Rep*. 2016;6:23561.
86. Andersson J, Vogt JK, Dalgaard MD, Pedersen O, Holmgaard K, Heegaard S. Ocular surface microbiota in patients with aqueous tear-deficient dry eye. *Ocul Surf*. 2021;19:210-217.
87. Chao C, Akileswaran L, Cooke Bailey JN, et al. Potential role of ocular microbiome, host genotype, tear cytokines, and environmental factors in corneal infiltrative events in contact lens wearers. *Invest Ophthalmol Vis Sci*. 2018;59:5752-5761.
88. Kittipibul T, Puangsricharern V, Chatsuwan T. Comparison of the ocular microbiome between chronic Stevens-Johnson syndrome patients and healthy subjects. *Sci Rep*. 2020;10:4353.
89. Prashanthi GS, Jayasudha R, Chakravarthy SK, et al. Alterations in the ocular surface fungal microbiome in fungal keratitis patients. *Microorganisms*. 2019;7:309.
90. Horai R, Caspi RR. Microbiome and autoimmune uveitis. *Front Immunol*. 2019;10:232.
91. Moon J, Choi SH, Yoon CH, Kim MK. Gut dysbiosis is prevailing in Sjogren's syndrome and is related to dry eye severity. *PLoS One*. 2020;15:e0229029.
92. Leonardi A, Modugno RL, Cavarzeran F, Rosani U. Metagenomic analysis of the conjunctival bacterial and fungal microbiome in vernal keratoconjunctivitis. *Allergy*. 2021;76:3215-3217.
93. Liang Q, Li J, Zhang S, et al. Characterization of conjunctival microbiome dysbiosis associated with allergic conjunctivitis. *Allergy*. 2021;76:596-600.
94. Cocks TM, Moffatt JD. Protease-activated receptor-2 (PAR2) in the airways. *Pulm Pharmacol Ther*. 2001;14:183-191.
95. Li B, Zou Z, Meng F, et al. Dust mite-derived Der f 3 activates a pro-inflammatory program in airway epithelial cells via PAR-1 and PAR-2. *Mol Immunol*. 2019;109:1-11.
96. Egawa G, Kabashima K. Barrier dysfunction in the skin allergy. *Allergol Int*. 2018;67:3-11.
97. Yeoh S, Church M, Lackie P, McGill J, Mota M, Hossain P. Increased conjunctival expression of protease activated receptor 2 (PAR-2) in seasonal allergic conjunctivitis: a role for abnormal conjunctival epithelial permeability in disease pathogenesis? *Br J Ophthalmol*. 2011;95:1304-1308.
98. Leonardi A, Brun P, Abatangelo G, Plebani M, Secchi AG. Tear levels and activity of matrix metalloproteinase (MMP)-1 and MMP-9 in vernal keratoconjunctivitis. *Invest Ophthalmol Vis Sci*. 2003;44:3052-3058.
99. Leonardi A, Sathe S, Bortolotti M, Beaton A, Sack R. Cytokines, matrix metalloproteases, angiogenic and growth factors in tears of normal subjects and vernal keratoconjunctivitis patients. *Allergy*. 2009;64:710-717.
100. Jung SJ, Mehta JS, Tong L. Effects of environment pollution on the ocular surface. *Ocul Surf*. 2018;16:198-205.
101. Leonardi A, Lanier B. Urban eye allergy syndrome: a new clinical entity? *Curr Med Res Opin*. 2008;24:2295-2302.
102. Torricelli AA, Matsuda M, Novaes P, et al. Effects of ambient levels of traffic-derived air pollution on the ocular surface: analysis of symptoms, conjunctival goblet cell count and mucin 5AC gene expression. *Environ Res*. 2014;131:59-63.
103. Gao ZX, Song XL, Li SS, et al. Assessment of DNA damage and cell senescence in corneal epithelial cells exposed to airborne particulate matter (PM2.5) collected in Guangzhou, China. *Invest Ophthalmol Vis Sci*. 2016;57:3093-3102.
104. DelMonte DW, Kim T. Anatomy and physiology of the cornea. *J Cataract Refract Surg*. 2011;37:588-598.
105. Park EJ, Chae JB, Lyu J, et al. Ambient fine particulate matters induce cell death and inflammatory response by influencing mitochondria function in human corneal epithelial cells. *Environ Res*. 2017;159:595-605.
106. Fujishima H, Satake Y, Okada N, Kawashima S, Matsumoto K, Saito H. Effects of diesel exhaust particles on primary cultured healthy human conjunctival epithelium. *Ann Allergy Asthma Immunol*. 2013;110:39-43.
107. Lasagni Vitar RM, Tau J, Janezic NS, et al. Diesel exhaust particles (DEP) induce an early redox imbalance followed by an IL-6 mediated inflammatory response on human conjunctival epithelial cells. *Exp Eye Res*. 2018;171:37-47.
108. Jin M, Wang Y, An X, et al. Phenotypic and transcriptomic changes in the corneal epithelium following exposure to cigarette smoke. *Environ Pollut*. 2021;287:117540.
109. Dogru M, Kojima T, Simsek C, Tsubota K. Potential role of oxidative stress in ocular surface inflammation and dry eye disease. *Invest Opthalmol Vis Sci*. 2018;59:DES163-DES168.
110. Dadaci Z, Oncel M, Oncel Acir N, Sahin E, Borazan M. Oxidative stress parameters and serum magnesium levels in patients with seasonal allergic conjunctivitis. *Cutan Ocul Toxicol*. 2016;35:270-274.
111. Zicari AM, Brindisi G, De Castro G, Lollobrigida V, Nebbioso M, Duse M. Is oxidative stress involved in vernal keratoconjunctivitis? Results from a pilot study in children. *Pediatr Allergy Immunol*. 2020;31(Suppl 26):52-56.
112. Fukase S, Ando T, Matsuzawa M, et al. Pollen shells and soluble factors play non-redundant roles in the development of allergic conjunctivitis in mice. *Ocul Surf*. 2021;22:152-162.
113. Chen W, Hu J, Zhang Z, et al. Localization and expression of zonula occludins-1 in the rabbit corneal epithelium following exposure to benzalkonium chloride. *PLoS One*. 2012;7:e40893.
114. Jia Z, Wang X, Wang X, et al. Calycosin alleviates allergic contact dermatitis by repairing epithelial tight junctions via down-regulating HIF-1alpha. *J Cell Mol Med*. 2018;22:4507-4521.
115. Sirin Kose S, Atakul G, Asilsoy S, Karaman O, Uzuner N, Anal O. Efficacy of allergen-blocker mechanical barrier gel on symptoms and quality of life in patients with allergic rhinitis. *Eur Arch Otorhinolaryngol*. 2019;276:729-734.
116. Ramesan VS, Jain S. Chitosan-glycerol gel as barrier formulation for metal allergy. *ACS Omega*. 2019;4:5900-5903.

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