Functions of ocular surface mucins in health and disease

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Abstract

Purpose of review—The purpose of the present review is to describe new concepts on the role of mucins in the protection of corneal and conjunctival epithelia and to identify alterations of mucins in ocular surface diseases.

Recent findings—New evidence indicates that gel-forming and cell surface-associated mucins contribute differently to the protection of the ocular surface against allergens, pathogens, extracellular molecules, abrasive stress, and drying.

Summary—Mucins are high molecular weight glycoproteins characterized by their extensive O-glycosylation. Major mucins expressed by the ocular surface epithelia include cell surface-associated mucins MUC1, -4 and -16, and the gel-forming mucin MUC5AC. Recent advances using functional assays have allowed the examination of their roles in the protection of corneal and conjunctival epithelia. Alterations in mucin and mucin O-glycan biosynthesis in ocular surface disorders, including allergy, non-autoimmune dry eye, autoimmune dry eye, and infection, are presented.

Keywords

gel-forming mucin; cell surface-associated mucin; O-glycan; ocular surface epithelia; ocular allergy; dry eye

Introduction

The stratified, non-keratinized epithelia of the cornea and conjunctiva are continuously exposed to allergens, debris, pathogens, desiccation, injury, and rubbing [1]. Ocular surface mucins play a critical role in the protection of these epithelia. In this review, we summarize data indicating that the two classes of mucins known, secreted (both gel-forming and small soluble) and cell surface-associated, have heterogeneous functions at the ocular surface. The functions of secreted mucins include: (i) clearance of allergens, pathogens and debris, (ii) lubrication, (iii) and antimicrobial activity. The functions of cell surface-associated mucins include: (i) surface protection against abrasive stress (boundary lubrication), (ii) formation of an apical cell surface barrier, (iii) and osmosensing. In addition, both secreted and cell surface-associated mucins contribute through their hydrophilic O-glycans, to the hydrophilic character of wet-surfaced epithelia.

Definition, structure and classification of mucins

Mucins are the largest and most highly glycosylated glycoproteins known. They are characterized by the presence of multiple tandem repeats of amino acids rich in serine and threonine in the central domain of the mucin core peptide, which provide sites for O-

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glycosylation [2]. The number of amino acids per tandem repeat varies between different mucin genes, and the number of tandem repeats per allele is also variable, making mucin genes and the resultant proteins polymorphic [3,4]. The extensive O-glycosylation of mucins begins with the enzymatic addition of N-acetyl-galactosamine (GalNAc) to serine or threonine on the tandem repeats by a family of glycosyltransferases called polypeptide GalNAc transferases (pp-GalNAc-Ts) [5]. Elongation of the O-glycan chain by sequential addition of carbohydrates is obtained through the activity of different glycosyltransferases, that are cell-type and cell-tissue specific. As a result of the extensive O-glycosylation, approximately 55% of the ocular mucin mass is composed of O-linked glycans [6]. O-glycans also contribute to providing an extended or "bottle brush-like" conformation to the mucin protein backbone [7].

Epithelial mucins can be divided in two different classes, cell surface-associated and secreted (Figure 1). So far, 11 cell surface-associated (MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20, MUC21) and 7 secreted mucins have been identified, the latter being further divided into two subcategories, soluble (MUC7, MUC9) and gel-forming (MUC2, MUC5AC, MUC5B, MUC6, MUC19) [8–11]. Cell surface-associated mucins are single-pass transmembrane proteins characterized by a short cytoplasmic tail, a transmembrane domain, an autoproteolytic domain called SEA module, and an extended highly glycosylated extracellular domain that contributes to the formation of the glycocalyx of apical cells in wet-surfaced epithelia. Gel-forming mucins are characterized by a large central tandem repeat domain flanked by cystein-rich domains that have homology to the so-called D domains of the von Willebrand factor [2]. These domains allow polymerization of individual mucin molecules through disulfide bond formation [10]. Soluble mucins are the smallest mucins known. They lack the cysteine-rich domains and are, therefore, found as monomers.

Distribution of mucins at the ocular surface epithelia

Gel-forming, cell surface-associated and small soluble mucins have been identified at the ocular surface epithelia. They are distributed throughout the corneal and conjunctival epithelia, goblet cells, and the lacrimal apparatus.

Corneal and conjunctival epithelia

The stratified corneal and conjunctival epithelia are known to express at least three membrane-associated mucins: MUC1, MUC4, and MUC16 [12–14]. These mucins are concentrated on the tips of the apical cells' microplicae, forming a dense glycocalyx at the epithelial-tear film interface, but can also be released from the cell surface and are found in the tear film [15]. MUC1 and MUC16 are present on both corneal and conjunctival epithelia, and are known to interact, through their cytoplasmic tails, with proteins in the cytoskeleton [16**,17]. MUC4 is most prevalent in conjunctival epithelium, with an apparent diminution toward the central corneal epithelium [18]. Conjunctival epithelium also expresses the small, soluble MUC7 [19].

Conjunctival goblet cells

Goblet cells are specialized cells intercalated in conjunctival epithelium the highest number of these being found in the infero-nasal portion of the bulbar conjunctiva [20]. They produce secretory granules that contain the gel-forming mucin MUC5AC [14,21]. Recent data obtained by immunofluorescence microscopy have also shown that MUC19 is present in conjunctival goblet cells; however, this has not yet been confirmed by in situ hybridization [22]. The gel-forming mucin MUC2 has also been detected in conjunctiva, but no data has yet been published to demonstrate goblet cell-specific localization [23].

Lacrimal apparatus

In the lacrimal gland, MUC1, MUC4, MUC16, MUC5AC, MUC5B, MUC6, and MUC7, have been detected [24,25]. MUC16 has also been detected in the accessory lacrimal glands of Krause and in the nasolacrimal ducts [24,26]. MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC6, and MUC7 are expressed by epithelia of the lacrimal sac and nasolacrimal ducts [26].

Function of mucins in the ocular surface epithelia

Traditionally, mucin function at the ocular surface has been ascribed to secreted gel-forming mucins acting as lubricating agents and clearing molecules. New evidence shows a role for cell surface-associated mucins in providing barrier function to corneal and conjunctival epithelia (Figure 2). This section will discuss data on the heterogeneous functions of secreted and cell surface-associated mucins at the ocular surface.

Secreted mucins

Clearing molecules—Gel-forming mucins have the capability to trap allergens and debris in order to facilitate their clearance from mucosal surfaces [27]. Electron microscopy and western blot analysis suggest that at the ocular surface these mucins tend to form polymers in the goblet cells, where they are stored, but are secreted as monomers in the tear film [15,28]. The presence of a low molecular weight mucin in tears may reflect the unique requirements of the ocular surface, where a polymerized, viscous coating of corneal and conjunctival epithelia would impair transparency and be detrimental for refractive purposes.

Gel-forming mucins can also act as counter receptors for pathogens and, therefore, contribute to their clearance from mucosal surfaces [27]. In animal models it has been shown that mucins in the tear film bind to *Pseudomonas aeruginosa*, thus preventing binding of the microorganism to the corneal epithelium and facilitating its clearance through the lacrimal drainage system [29].

Lubricating agents—Gel-forming mucins, through their ability to retain water, can form a highly hydrated mucus gel and contribute to the lubrication of epithelial surfaces [30]. The highly hydrophilic character of secreted mucins may be responsible for lubrication of the ocular surface and reduction of shear stress during blinking or rubbing.

Antimicrobial agents—It has been shown that a 20-mer peptide of the small, soluble mucin MUC7 has potent antifungal and antimicrobial activity in the salivary gland [31]. Although MUC7 has not been detected in tears to date [15], it is possible that it also acts as an antimicrobial agent in the lacrimal gland.

Cell surface-associated mucins

Disadhesive molecules—Cell surface-associated mucins form a thick cell surface glycocalyx that may extend up to 500 nm from the plasma membrane at the ocular surface [32]. Using static and dynamic flow assays, membrane-bound mucins have recently been shown to provide through their O-glycans a disadhesive character to the apical surface of corneal epithelial cells [33**].These results suggest that cell surface-associated mucins provide boundary lubrication and prevent adhesion of facing cell surfaces (i.e. corneal epithelium and tarsal conjunctiva) during blinking or sleeping.

Protective cell surface barrier—Recent evidence has shown that cell surface-associated mucins can contribute to the maintenance of the mucosal barrier integrity, preventing the penetrance of extracellular molecules onto ocular surface epithelia. In human corneal epithelial

cells, the expression of membrane-bound mucins prevents penetrance of rose bengal, an anionic, organic dye used in clinical practice to evaluate epithelial damage in diseases such as dry eye [16,34]. The role of cell surface-associated mucins in providing a protective cell surface barrier has also been shown in other wet-surfaced epithelia, such as those in the gastro-intestinal tract, where abrogation of mucins and mucin O-glycans resulted in impaired mucosal integrity as shown by increased permeability to FITC-dextran, development of colitis, and systemic infection [35**,36**].

Osmosensors—Recent data from studies in yeast have shown that the mucin-like transmembrane proteins Hrk1 and Msb2 can activate the HOG signaling pathway in response to increased osmolarity [37**]. It has, therefore, been hypothesized that membrane-bound mucins in higher eukaryotic cells could have the novel function of sensing the extracellular environment through their extracellular domain, and signal through their intracellular domain [38].

Alteration of mucins in ocular surface disease

As described above, mucins are essential to maintain proper epithelial protection, and therefore a healthy wet-surface phenotype. Examples of diseases in which mucin biosynthesis and mucin O-glycosylation are altered at the ocular surface are described in this section, and include allergy, non-autoimmune dry eye, autoimmune dry eye, and infection.

Ocular allergies

Allergic diseases of the ocular surface in which alterations in both cell surface-associated and secreted mucins have been described include atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC). AKC is a disease characterized by squamous metaplasia of the conjunctival epithelium and goblet cell loss. Patients with AKC have decreased levels of the goblet cell-specific mucin MUC5AC, which has been linked to loss of lubrication and epithelial damage [39,40]. Concomitant to the decrease in MUC5AC, there is an increase in the expression of MUC1, MUC2 and MUC4, which may represent a defense mechanism to compensate for the loss of protection provided by the goblet cell mucin MUC5AC [39].

VKC is a disease characterized by seasonal or perennial allergy symptoms that exacerbate in the spring, with intense itching, photophobia, and mucous discharge. Patients with VKC have increased numbers of conjunctival goblet cells and increased levels of MUC5AC, suggesting a protective mechanism aimed at clearing allergens from the ocular surface [41,42]. Active phases of the disease are characterized by intense ocular surface inflammation and eosinophilic infiltration [42]. Activated eosinophils are known to increase MUC5AC expression [43] and, therefore, could contribute to the mucus discharge observed in these patients. Following topical anti-allergic and anti-inflammatory treatments in VKC patients, there are reductions in conjunctival goblet cell number and MUC5AC expression towards normal levels [44,45].

Animal models have also been used to study the expression of mucins after application of defined allergens. In a mouse model of allergic conjunctivitis, repetitive application of cat dander or peptide P3-1 initially induced a decrease in the number of filled goblet cells, as well as a reduction in Muc5AC and Muc4 mRNA expression. After 24 to 48 hours, goblet cell numbers and mucin expression levels returned to normal levels [46], indicating that goblet cell differentiation and increased mucin secretion act as a defense mechanism to remove allergens.

Non-autoimmune dry eye

Dry eye, or keratoconjunctivitis sicca, is a multifactorial disease of the ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential

damage to the ocular surface [47]. Dry eye can be divided in two types: aqueous-deficient including Sjögren's and non-Sjögren's and evaporative. In this section we will discuss non-Sjögren's and evaporative dry eye.

Early studies on dry eye patients found alterations in carbohydrate content within goblet cells. Using gold-conjugated lectins, Versura et al. showed a decrease in sialic acid and GalNAc in secretory granules of goblet cells of patients with dry eye [48], suggesting that alterations in the glycosylation of gel-forming mucins may be responsible for the loss of hydrophilicity and lubrication, as well as a reduction in tear film stability. More recent studies on cell surface-associated mucins have shown that their carbohydrate component is also altered in dry eye. By immunofluorescence microscopy on conjunctival impression cytology samples, binding of the H185 antibody to an O-acetyl sialic acid epitope on MUC16 was altered on the apical cell surface of non-Sjögren's dry eye patients [49,50]. In normal conjunctiva, the antibody bound apical epithelial cell membranes, whereas in dry eyes, a reduction of apical cell binding was observed concomitant to rose bengal staining of the conjunctival epithelium. Therefore, it is possible to speculate that alteration of mucin O-glycosylation in dry eye compromises the ocular surface epithelial barrier and results in increased abrasive stress and epithelial damage.

Dry eye is also a common feature of endocrine diseases. In complete androgen insensitivity syndrome a rare disease characterized by complete androgen deficiency due to mutations in the gene coding for the androgen receptor a significant reduction in the expression of both MUC1 and MUC5AC associated with dryness and reduced tear film stability, have been reported at the ocular surface [51*]. On the other hand, patients with polycystic ovary syndrome a common disease in reproductive age women characterized by hyperandrogenism show mucous discharge associated with reduced tear film stability and evaporative dry eye [52]. These patients have an increased goblet cell number and MUC5AC mRNA expression, which may contribute to the abnormal mucous discharge.

Lastly, one of the main features of dry eye syndrome is an increased tear film osmolarity [47]. Recent studies in yeast on mucin-like proteins Hkr1 and Msb2 have shown that membrane-bound mucins could also display features of osmosensors triggering signaling cascades [37**,38]. This possibility has yet to be confirmed in mammalian epithelia; however, it is possible to speculate that in dry eye, cell surface-associated mucins could function as osmosensors to induce signaling responses.

Autoimmune dry eye

Autoimmune diseases in which alterations of ocular surface mucins have been described include Sjögren's syndrome and ocular cicatricial pemphigoid (OCP).

Sjögren's syndrome is a systemic autoimmune disease most prevalent in women in their fourth and fifth decades of life. It is characterized by dryness of eyes, mouth, and other mucosae. At the ocular surface of patients affected by Sjögren's dry eye, decreased levels of the goblet cell mucin MUC5AC have been detected in conjunctival epithelium and in tears [53], which correlates with decreased number of conjunctival goblet cells. Decreased levels of MUC19, another recently discovered gel-forming mucin, have also been described in conjunctival epithelium of patients with Sjögren's dry eye [22].

OCP is another systemic autoimmune disease that causes chronic cicatrizing conjunctivitis, progressive subepithelial fibrosis of the conjunctiva and, in late stages, complete keratinization and drying of ocular surface epithelia. It has been reported that the cell-layer and cell-type-specific distribution of conjunctival pp-GalNAc-Ts is altered in these patients [54]. In early stages of OCP, there is an overproduction of pp-GalNAc-T isoenzymes on apical cells of the ocular surface, suggesting an early compensatory attempt to synthesize mucin O-glycans to

maintain a wet-surfaced phenotype. This early increase in pp-GalNAc-Ts in the apical layers is reduced as keratinization and drying of the ocular surface proceeds [54].

Infectious diseases

It has been proposed that cell surface-associated mucins create a physical barrier against infection on the ocular surface epithelia, although to date no data exists with regard to human ocular surface disease. In Muc1-null mice, inoculation of the ocular surface with Corynebacteria and coagulase-negative Staphylococci increased blepharitis and conjunctival infection [55]. However, these results contrast with a later study investigating adherence of *Pseudomonas aeruginosa* to the ocular surface epithelia of Muc1-null mice, which showed no increase in bacterial adherence and conjunctivitis [56]. This discrepancy could be related to mouse strain variation in response to deletion of Muc1, to strain variation of pathogens in the two studies, or to different animal housing settings (in the first study, a conventional animal facility was used, while a murine pathogen-free environment was used in the second study). A recent paper describing increased binding of *Staphylococcus aureus* to corneal limbal epithelial cell cultures lacking MUC16, supports a role for cell surface-associated mucins in creating a physical barrier against pathogen penetrance [16**].

Conclusions

Mucins are hydrophilic molecules essential for the homeostasis of wet-surfaced epithelia. In this review, we show evidence that mucins could play multiple roles in the protection of mucosal surfaces. Gel-forming mucins are essential for lubrication and clearance of allergens, pathogens and debris. Cell surface-associated mucins provide the epithelial surface with an anti-adhesive character essential to reducing abrasive stress and could form a protective apical barrier against microorganisms and extracellular molecules.

At the ocular surface, alteration of mucin expression and glycosylation are associated with a number of diseases. Recent advances in understanding the role of mucins in maintaining an intact mucosal barrier could contribute to the development of new therapeutic strategies.

Acknowledgements

Abbreviations

AKC
atopic keratoconjunctivitis

FITC
fluorescein isothiocyanate

GalNAc
N-acetyl-galactosamine

HOG
high osmolarity glycerol

MUC
human mucin

Muc
mouse mucin

OCP

> ocular cicatricial pemphigoid

pp-GalNAc-Ts

> polypeptide N-acetyl-galactosamine transferases

SEA

> sea-urchin sperm protein, enterokinase and agrin

VKC

> vernal keratoconjunctivitis

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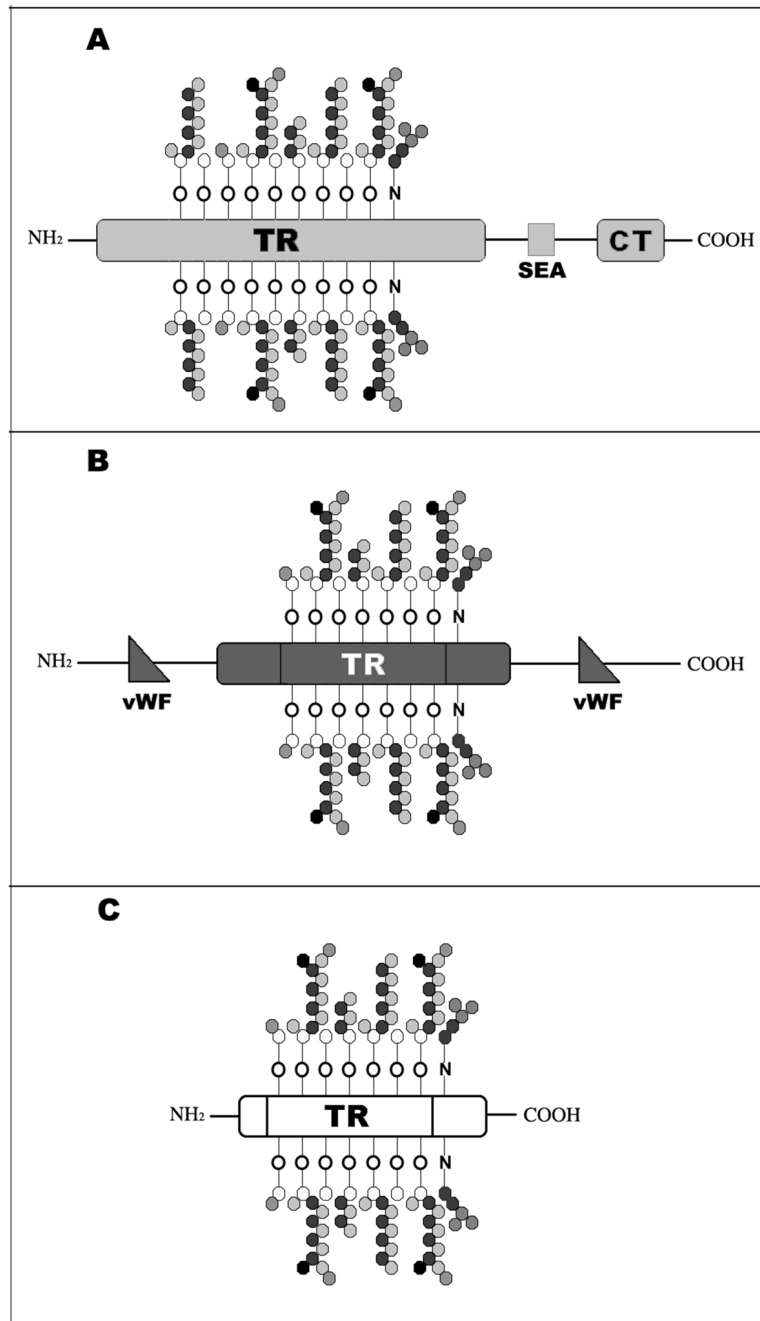
Figure 1. Structure of mucins
Mucins are high molecular weight glycoproteins characterized by a variable number of heavily
O-glycosylated Tandem Repeats (TR) rich in serine and threonine. Cell surface-associated
mucins (**A**) are anchored to the cell membrane and have a small cytoplasmic tail (CT). At the
ocular surface, MUC1 and MUC16 contain the SEA autoproteolytic domain. Gel-forming
mucins (**B**) are the largest glycoproteins known and can form polymers through their cystein-
rich domains (von Willebrand factor-like domains, vWF). Soluble mucins (**C**) are the smallest
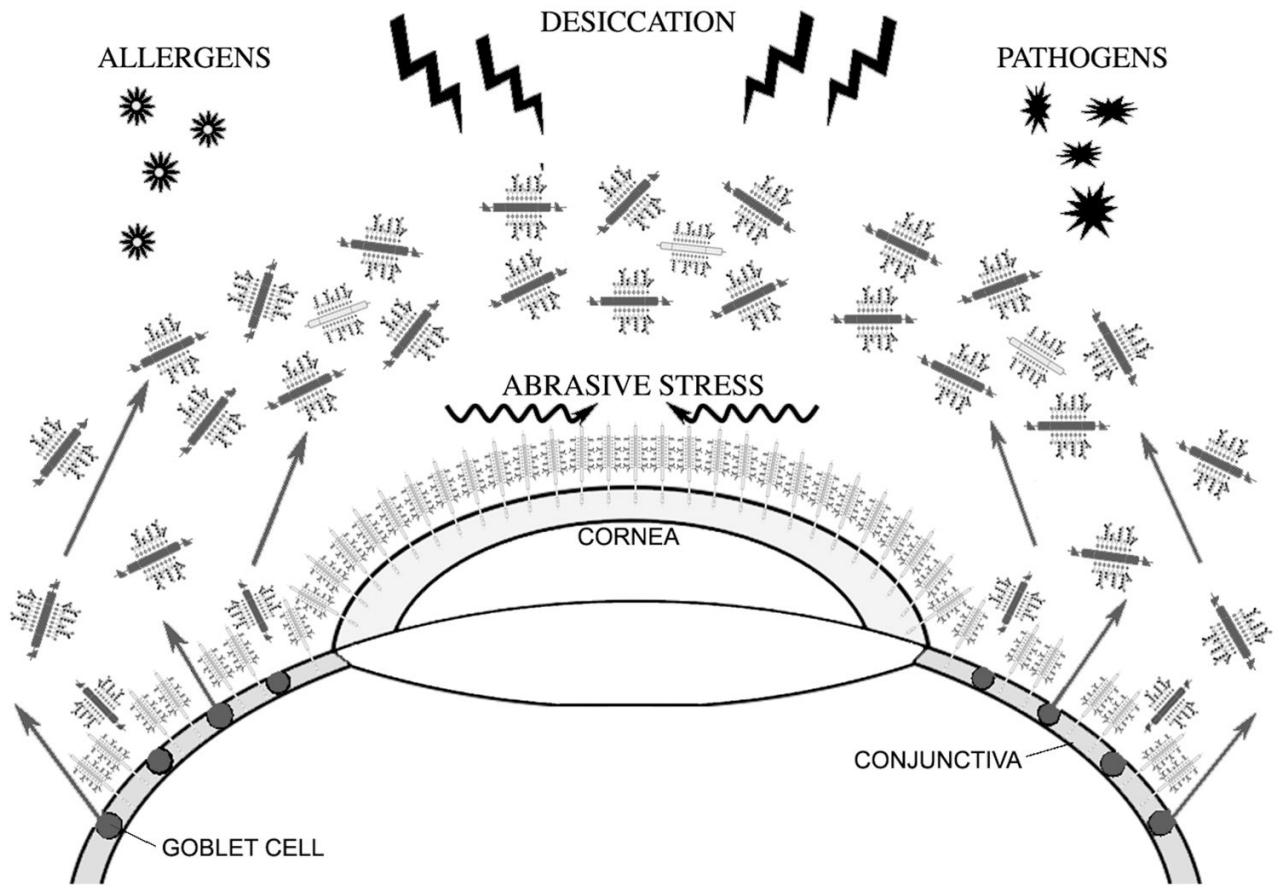mucins known and lack both the transmembrane domain and the vWF domains.

Figure 2. Proposed roles of mucins at the ocular surface
Gel-forming mucins in the tear film, together with cell surface-associated mucins lining the epithelial glycocalyx, contribute to the formation of a protective barrier at the ocular surface. Gel-forming mucins lubricate the ocular surface and allow clearing of allergens and pathogens. Cell surface-associated mucins reduce abrasive stress and could form an apical barrier to pathogens and extracellular molecules. Through their hydrophilic O-glycans, mucins also contribute to the prevention of epithelial desiccation.