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Reconsidering the central role of mucins in dry eye and ocular surface diseases

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ABSTRACT

Mucins are key actors in tear film quality and tear film stability. Alteration of membrane-bound mucin expression on corneal and conjunctival epithelial cells and/or gel-forming mucin secretion by goblet cells (GCs) promotes in ocular surface diseases and dry eye disease (DED). Changes in the mucin layer may lead to enhanced tear evaporation eventually contributing to tear hyperosmolarity which has been associated with ocular surface inflammation. Inflammatory mediators in turn may have a negative impact on GCs differentiation, proliferation, and mucin secretion. This sheds new light on the position of GCs in the vicious circle of DED. As contributor to ocular surface immune homeostasis, GC loss may contribute to impaired ocular surface immune tolerance observed in DED. In spite of this, there are no tools in routine clinical practice for exploring ocular surface mucin deficiency/dysregulation. Therefore, when selecting the most appropriate treatment options, there is a clear unmet need for a better understanding of the importance of mucins and options for their replacement. Here, we comprehensively revisited the current knowledge on ocular surface mucin biology, including functions, synthesis, and secretion as well as the available diagnostic tools and treatment options to improve mucin-associated homeostasis. In particular, we detailed the potential link between mucin dysfunction and inflammation as part of the uncontrolled chronic inflammation which perpetuates the vicious circle in DED.

1. Introduction

Dry eye disease (DED) was recently re-defined as *"a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles"* (Craig et al., 2017). DED is a common heterogeneous disease associated with considerable variability in clinical settings. Although DED usually appears to be mild to moderate in terms of ocular surface damage, it may have significant impact on quality of life and vision threat, especially in the most severe cases. The causes of DED are multiple and the disease often involves several mechanisms, which may be interdependent, including lacrimal secretion insufficiency, meibomian gland dysfunction (MGD), corneal nerve impairment and mucin layer alterations (Baudouin et al., 2014, 2018, Bron et al., 2017). In addition, various environmental and non-environmental risk factors such as age, sex, autoimmunity, drying systemic medication, and desiccating environment stress contribute to the disease (Craig et al., 2017). As a consequence of this multifactorial pathophysiology, DED cannot be characterized by a single mechanism, sign or symptom and it remains difficult to determine disease severity, to monitor disease progression or to assess response to treatment in

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everyday clinical practice, mainly due to a lack of correlation between symptoms and clinical signs (Nichols et al., 2004). Some attempts have been made to standardize the detection of the most severe cases (Baudouin et al., 2014) but a global approach of the different stages remains to be agreed upon the assessment of disease severity, and in this respect, the consequences of mucin abnormalities should be taken into consideration.

Mucins are large extracellular glycoproteins which cover most mucosal surfaces in the body. They are the main component of mucus, an adhesive viscoelastic gel which functions to maintain a healthy wet surface and to form a barrier against pathogens and other environmental toxic agents (Cone, 2009). At the ocular surface, mucins are secreted by conjunctival goblet cells (GCs) and lacrimal glands, and are also expressed at the apical membrane of the squamous corneal and conjunctival epithelia (Gipson, 2004). As experimentally suggested by Dilly (Dilly, 1994), membrane-bound mucins at the surface of upper epithelial cells form the deepest compartment of the tear film, while secreted mucins diffuse in the aqueous layer, from the glycocalyx to the lipid layer, following a gradient that forms a muco-aqueous phase. This model is now commonly accepted (Willcox et al., 2017).

Both secreted and membrane-bound mucins are essential to maintain wettability of the ocular surface and thereby contribute to sustain tear film dynamics, stability, osmolarity and homeostasis. Some of them, such as the gel-forming mucin MUC5AC also play a protective role in trapping and clearing cellular debris and foreign bodies including allergens and pathogens (Ablamowicz and Nichols, 2016). Thus, alterations in the structure and/or expression pattern of mucins are related to the pathogenic processes of various ocular surface diseases, including DED and ocular allergy.

Regarding DED, despite the multitude of the underlying causes, there are several common histopathologic manifestations of the ocular surface epithelia including the loss of conjunctival GCs, abnormal enlargement of the epithelial cells (squamous metaplasia), increase in cellular stratification, and keratinization (Tseng et al., 1984). There is a body of evidence that suggests GC loss may be directly related to chronic inflammation and cell surface apoptosis subsequent to cell hyperosmolarity and chronic damage, resulting in further in tear film instability. Conversely mucus deficiency can sustain hyperosmolarity and inflammation in a vicious circle (Baudouin et al., 2013, 2018). It has also been reported that GCs may play a role in innate immunity at the ocular surface (Contreras-Ruiz and Masli, 2015). Although mucin deficiency may be considered both a cause and a consequence of DED, it is not currently investigated in routine clinical practice. The purpose of this review was thus to revisit the concept of mucin and ocular surface interactions, by providing a comprehensive overview of the current knowledge of secreted and membrane-bound mucin biology, including functions, synthesis, and secretion as well as the available diagnostic tools. Identification of mucin deficiency and characteristics could help in the selection of appropriate treatment options to restore mucin-associated homeostasis.

2. Mucin biology and functions

2.1. Mucins characteristics

Mucins (MUC) comprise a family of large, highly glycosylated, hydrophilic proteins which are extremely heterogeneous in terms of molecular weight. The hallmark of these glycoproteins is their long peptide chain composed of multiple short tandem repeats of amino acids, rich in serine and threonine. Each serine and threonine residues provides a site for O-linked glycosylation. O-glycosylation is initiated by the enzymatic addition of N-acetyl-galactosamine (GalNAc) to the hydroxyl group side of serine and threonine residues. 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 (for review see Guzman-Aranguez and Argüeso, 2010). As a result of the extensive O-glycosylation, the mucin carbohydrate chains can provide 50–90% of the molecular mass of the glycoprotein. To date, more than 20 mucin genes have been identified, and up to 10 mucins are expressed at the ocular surface (Spurr-Michaud et al., 2007).

Table 1
Ocular mucins type and location.

	Type of mucins	Ocular location	Identified in tears
MUC1	Transmembrane	Lacrimal glands Apical corneal and conjunctival epithelial cells	Yes (Extracellular domain)
MUC2	Gel-forming	Goblet cells	Yes
MUC4	Transmembrane	Cornea, conjunctiva, lacrimal glands Apical conjunctival epithelial cells	Yes (Extracellular domain)
MUC5AC	Gel-forming	Apical surface of GCs Lid wiper	Yes
MUC7	Soluble	Lacrimal glands, Stratified epithelium	No
MUC13	Transmembrane		No
MUC15	Transmembrane		No
MUC16	Transmembrane	Apical corneal epithelial surface. Lacrimal gland ductal epithelial cells	Yes (Extracellular domain)
MUC17	Transmembrane		No
MUC19	Gel-forming	Goblet cells	No
MUC20	Transmembrane	Basal and intermediate epithelial cell layer	No

Mucins are generally categorized into three distinct families: transmembrane, gel-forming, and soluble (Gipson, 2004) (Table 1). The membrane-bound mucins are monomeric mucins with a short cytoplasmic tail and a transmembrane domain embedded in the lipid bilayer of epithelial cells. MUC1, MUC4, and MUC16 are the main transmembrane mucins expressed at the ocular surface. They are expressed by stratified squamous epithelia along apical membranes of the apical surface of the corneal and conjunctival stratified epithelia at the tips of microplicae (Gipson, 2004). MUC1, MUC4 and MUC16 are expressed by corneal and conjunctival epithelial cells (Inatomi et al., 1995; Inatomi et al., 1996), and a greater levels of MUC4 transcripts has been detected in the peripheral than in the central corneal epithelium (Pflugfelder et al., 2000). The alpha-domain of membrane-bound MUC1, MUC4 and MUC16 can be constitutively shed from the apical membrane of epithelial cells leading to soluble mucins released into the tear film. The proteolytic cleavage mechanism is not well known but might involve inflammatory mediators and metalloproteinases (Blalock et al., 2008). MUC20 is another transmembrane mucin strongly expressed at the ocular surface throughout the cornea and conjunctiva, but predominantly in the intermediate cell layer of the stratified epithelia with limited expression on the apical glycocalyx (Woodward and Argüeso, 2014). Transcripts of three other transmembrane mucins, MUC13, MUC15, and MUC17, have also been detected in normal conjunctiva (Corrales et al., 2003), but their expression and function are less well known.

Gel-forming mucins (MUC5AC, MUC2, and MUC19) are characterized by various cysteine-rich domains (D domains) located at both the N and C terminus of the mucin backbone and are required for homomultimerization and gel formation (Gipson, 2004). MUC5AC is produced and secreted by epithelial GCs, and is the most abundant gel-forming mucin at the ocular surface (Hodge and Dartt, 2013). The gel-forming mucin MUC5AC has been consistently detected in human normal tears and precorneal tear film using different sampling methodologies and immunoassay (Spurr-Michaud et al., 2007; Ablamowicz and Nichols, 2017). MUC2 is another gel-forming mucin detected on the ocular surface and in tears (Spurr-Michaud et al., 2007), but substantially less expressed compared to MUC5AC. More recently, mRNA transcripts encoding a new gel-forming mucin, MUC19, and the

Table 2
Ocular mucins and main known functions.

Gel-forming mucin	MUC5AC	Clearance of contaminant Ocular surface hydration and lubrication Provide a smooth and refractive corneal surface Tear film stability
	MUC2, MUC19	Not known
Membrane-bound mucin	MUC1, MUC4, MUC16	Disadhesion Boundary lubrication Barrier function
	MUC20	Epithelium integrity
	MUC13, MUC15, MUC17	Not known
Soluble mucin	MUC7	Not known

corresponding product have been found in cornea, conjunctiva and lacrimal gland tissues (Yu et al., 2008).

MUC7 is a soluble monomeric secreted mucin that lacks both the transmembrane domain and cysteine-rich domains (Corrales et al., 2003) and thus cannot form gel like MUC5AC. MUC7 is mainly secreted by acinar cells of the lacrimal glands but also by conjunctival epithelial squamous cells. Studies have failed to detect MUC7 in tears (Jumblatt et al., 2003; Spurr-Michaud et al., 2007) and its exact ocular function is not well known.

In summary, current knowledge indicates that the mucin component in tears is a mixture of secreted (mainly MUC5AC but also low levels of MUC2) and shed membrane-bound soluble mucins (MUC1, MUC4, and MUC16) (Spurr-Michaud et al., 2007).

2.2. Mucin functions

As described above, there are a variety of mucins which differ in their ocular surface distribution, size, and structural motif. Both membrane-bound and gel-forming mucins play various functions critical to the health of the ocular surface (Table 2). Other specific roles such as cell signaling have been suggested.

2.2.1. Hydrodynamic lubrication

It is currently accepted that the tear film is modeled as a two-phase model: a lipid layer overlying a muco-aqueous phase, and the muco-aqueous layer overlying the apical epithelial cells and their carbohydrate-rich glycocalyx. The aqueous and mucin layers are considered as a single layer of mucoaqueous gel (Willcox et al., 2017).

Gel formation of soluble mucins including membrane-shed and secreted mucins is achieved by the dual structural features of the mucus glycoproteins, i.e. a covalent polymeric structure of hydrophobic amino acid chains and non-covalent interactions between glycoprotein molecules ensuring relatively stable entanglement of carbohydrate side-chains (Sellers et al., 1991). This gel-like structure is extremely hydrophilic and hydroscopic leading to a desirable resistance to drainage and contributing to lubrication of epithelial surfaces, limiting frictional damage (Mantelli and Argüeso, 2008).

In addition, the gel-forming mucins provide the non-newtonian shear-thinning property of the tear film i.e. viscosity fall with increasing shear rate (shear thinning): when the eyelids are opened, the tear film is more viscous, and inversely, during blinks, the high shear applied by the eyelid breaks the weak interactions of the mucin network and the layer can flow more easily, thus preventing any damage that a constant high viscosity may cause to the underlying corneal epithelium (Tiffany, 1991). Factors which may modulate the mucin viscoelasticity include salt concentration, pH, mucin concentration, level of hydration, and trefoil factor (Demouveaux et al., 2018).

2.2.2. Aqueous tear film anchorage

Membrane mucins form a thick electron-dense glycocalyx (of about 500 nm) at the apical surface of corneal and conjunctival epithelial cells (Gipson, 2004; Cone, 2009). The glycocalyx expands along the ocular surface enlarged by the microvilli and microplicae structure of the stratified epithelial cells (Koufakis et al., 2006). This provides a relatively rigid structure at the ocular surface maintaining an adherent layer despite the vigorous and constant shearing actions of eye blinks (Cone, 2009). It was previously suggested that membrane mucins (such as MUC1) facilitate spreading of the MUC5AC-containing mucus on the ocular surface (Gipson and Inatomi, 1998).

Movement of the mucus layer over the glycocalyx implies that the mucins of the glycocalyx are disadhesive, which is ensured by repulsive negative charge interactions between secreted mucins and membrane-bound mucins (Sumiyoshi et al., 2008). This is also facilitated by the ability of the mucus to stretch and disentangle according to movement (Cone, 2009). In addition, membrane-associated mucins (MUC1, MUC16) can bind to galectin-3, a soluble β-galactoside-binding glycoprotein which can cross-link glycan ligands on cell surface receptors to generate molecular lattices (Argüeso et al., 2009). The resulting galectin-mucin interaction was suggested to help in mucin assembly on the epithelial cell surface (Argüeso et al., 2009, Gipson et al., 2014).

2.2.3. Mucins and tear film stability

Secreted mucins (soluble or membrane-shed) are believed to diffuse in the tear film following a gradient from the ocular surface to the lipid layer to form a muco-aqueous phase (Dilly, 1994; Willcox et al., 2017). Tear film stability is maintained by a balanced interaction between tear components including mucin and the lipid layer. It is now well established that hyperevaporation of the tear film is not only due to lipid layer abnormality but also to poor tear film spreading and increased surface tension. The presence of mucin in tears is believed to lower surface tension by interaction with tear lipids (Holly and Lemp, 1977; Corfield et al., 1997; Sweeney et al., 2013). Increased surface tension has been associated with low tear break-up time (TBUT) and it has been suggested that dry spot formation could be the result of a reduction in the critical surface tension at the ocular surface (Sweeney et al., 2013).

2.2.4. Clearance of contaminants and maintenance of epithelium integrity

Beside their hydrodynamic and tear film anchorage functions, mucins play a major role in trapping and clearing desquamated cells, cellular debris, foreign bodies, allergens and pathogens from the ocular surface preventing corneal damage and infection (Cone, 2009). This role is facilitated by the remarkable diversity of the carbohydrate side chains which enhances the possibility of pathogens binding to the mucus (Thornton and Sheehan, 2004). Energetic studies have shown that hydrophilic contaminants are easily repulsed by the mucus gel while weakly polar or apolar contaminants are trapped in the mucus gel (Sharma, 1993). Contaminants including pathogens and allergens are then eliminated through the nasolacrimal drainage system along with the mucus, contributing to tear renewal at every eye blink (Gipson and Argüeso, 2003; Dartt & Masli, 2014). In addition, membrane-associated mucin (MUC1, MUC16) probably plays a key role in maintaining mucosal barrier function through carbohydrate-dependent interactions with galectin-3. This interaction seems to prevent damage and infection of the ocular surface epithelium (Argüeso et al., 2009).

2.2.5. Other functions

Transmembrane mucins are also engaged in signal transduction, through extracellular domain-mediated ligand binding or by interacting with receptors for growth and differentiation factors, as reviewed by Singh & Hollingsworth (Singh and Hollingsworth, 2006). MUC1 contains multiple potential tyrosine phosphorylation sites which may be involved in the signaling pathways of growth factor receptors, interactions with ICAM and bacteria binding (Singh and Hollingsworth, 2006). MUC4 has extracellular EGF-like domains (Pflugfelder et al., 2000) which bind to the EGF receptors ErbB2 and ErbB3, two receptors that induce epithelial cell proliferation and apoptosis (Singh and

Hollingsworth, 2006). The MUC16 cytoplasmic domain binds to the actin cytoskeleton and interacts with specific proteins involved in the formation of surface membrane protrusion such as microvilli and microplicae, responsible for fluid layer stabilization (Govindarajan and Gipson, 2010). More recently, the cytoplasmic tails of MUC1, MUC13 and MUC16 have been reported to localize in the nucleus (van Putten and Strijbis, 2017), suggesting that the cytoplasmic tail of MUC1 could be released from the membrane to modulate the function of transcription factors and regulatory proteins. In an animal model of intestinal inflammation, MUC13 was shown to inhibit apoptosis and suppress inflammation. They suggested that damage to the epithelial barrier may lead to excessive shedding of mucin extracellular domains and subsequent activation of pathways linked to cell proliferation and apoptosis (van Putten and Strijbis, 2017).

2.3. Mucins expression and secretion

The amount of mucins produced is dependent upon regulation of mucin synthesis, mucin secretion, number of mucin producing cells, and mucin degradation (Hodges and Dartt, 2013).

2.3.1. Regulation of MUC5AC secretion

MUC5AC, the main gel-forming mucin at the ocular surface, is exclusively produced and secreted by epithelial GCs. GCs are highly polarized exocrine cells identified by their extensive apical accumulation of large secretory granules. Immature GCs present with a large nucleus whereas mature GCs have a small decentered nucleus and a large cytoplasm rich in secretory granules (Fig. 1). These cells are located in the superficial and intermediate cell layers, and their shape depends on their stage of secretion (Abdel Khalek et al., 1978). They are interspersed singly or in clusters throughout the stratified squamous cells of the conjunctiva but are absent from the limbus and corneal surface. They are connected by tight junctions to neighboring epithelial cells or other GCs (Gipson, 2016). In addition, they are not uniformly distributed throughout the ocular surface, but show regional variation; the medial fornices and palpebral region near the drainage system contain more GCs per unit area than the tarsal and bulbar conjunctiva. The inferior conjunctiva contains more GCs compared to the superior conjunctiva (Norn, 1958). GCs are also present in the inner eyelid borders (lid wipers) and within cryptal epithelial infoldings (Knop et al., 2012).

MUC5AC secretion is stimulated rapidly upon environmental stimuli including allergens, nerve stimulation, inflammatory mediators, or changes in temperature and osmolarity (Dartt and Masli, 2014; Gumus and Pflugfelder, 2017; Barbosa et al., 2017). GCs appear to continuously secrete MUC5AC using either a merocrine process in normal condition or an apocrine process in case of endogenous or exogenous irritation (Murube, 2012). Under normal conditions, mucin secretion by GCs is regulated by a neural reflex arising from sensory nerves on the cornea and conjunctiva that activates parasympathetic nerves surrounding the GCs. Muscarinic receptors (M1, M2, and M3) are expressed at the surface of GCs (Diebold et al., 2001) and cholinergic agonists and the vasointestinal peptide (VIP) induce conjunctival GC secretion (Dartt et al., 1996; Rios et al., 1999). Besides neuromediators, adenine nucleotides (UTP, ATP) are also potent stimulators of MUC5AC secretion through activation of P2Y2 purinergic receptors (Jumblatt and Jumblatt, 1998) as well as proinflammatory mediators including leukotrienes, histamine, and pro-inflammatory cytokines (Dartt and Masli, 2014).

2.3.2. Goblet cell proliferation and differentiation

Since all or most of secretory granules are discharged upon stimulation, this implies a rapid biosynthesis and/or GC renewal at the ocular surface. Biosynthesis of mature glycosylated mucin requires (a) transcription of a MUC gene to encode a MUC mRNA in the nucleus, (b) translation into MUC protein backbone on ribosome and protein folding in the endoplasmic reticulum, and c) posttranslational modification of mucin core proteins by glycosyltransferases in the Golgi apparatus (Hodges and Dartt, 2013).

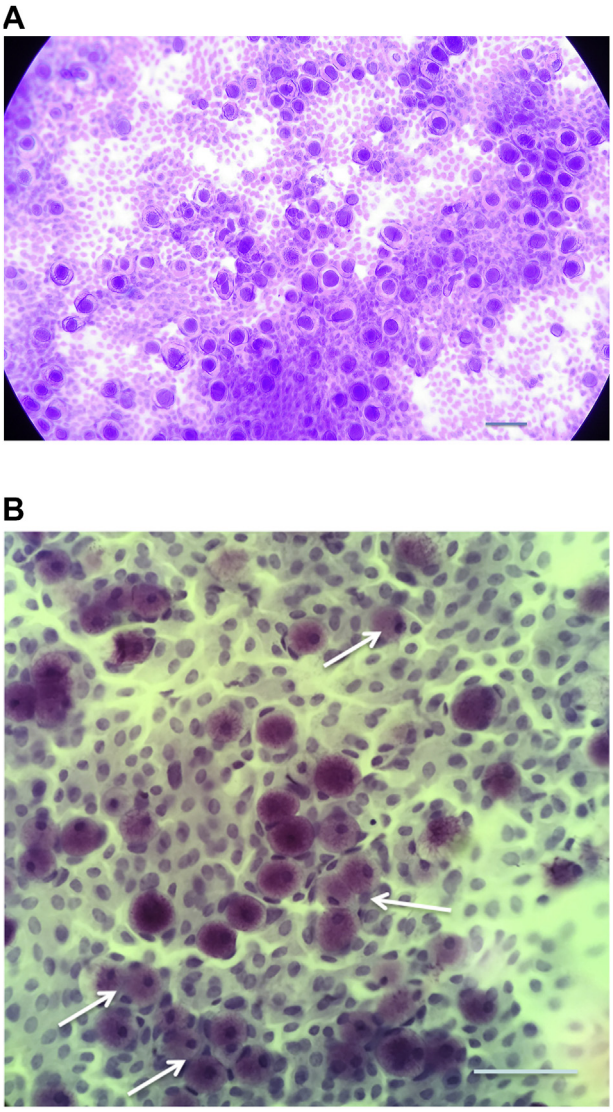

Fig. 1. Goblet cells in impression cytology specimens. A: immature GCs appear as large cells with a dense, centered nucleus and small cytoplasm. GCs are surrounded by epithelial cells. B: Mature GCs are large cells with small decentered, nuclei. Cytoplasmic mucins appear in pinkish color. Cresyl violet staining in impression cytology specimens (bar = 100 microns).

In normal conditions GCs are relatively quiescent, but there is a GC subpopulation which is able to proliferate and differentiate (Wei et al., 1995). In mouse and rabbit, there is some evidence that GCs and conjunctival epithelial cells arise from a common epithelial stem cell principally located in the fornix (Wei et al., 1997; Pellegrini et al., 1999; Ramos et al., 2015: Stewart et al., 2015). Primary cultures of GCs can therefore be obtained from the fornix but also the perilimbal conjunctiva (Shatos et al., 2008) (Fig. 2). Clonal analysis of human conjunctival epithelium shows that GCs differentiate from epithelial cells after a programmed number of cell doubling (Pellegrini et al., 1999); however, there is little information regarding the factor(s) controlling the differentiation pathways (Marko et al., 2013). Transcriptional events regulating GC differentiation involve the action of the Notch and Wnt cascades and the activation of the “sterile alpha motif” (SAM) pointed domain containing Ets transcription factor (Spdef) (Gipson, 2016). Spdef-deficient mice completely lack GCs, leading to a dry eye phenotype with corneal barrier disruption, conjunctival infiltration by

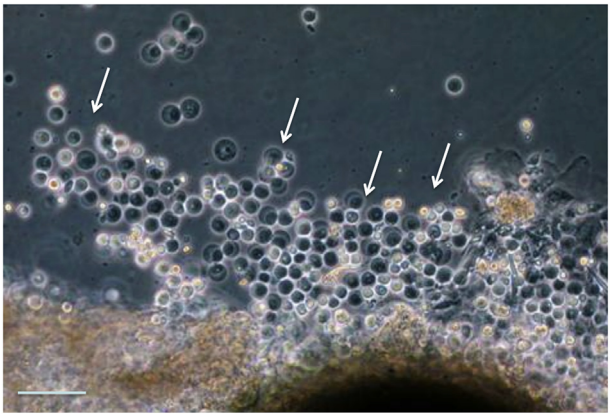

Fig. 2. Primary culture of goblet cells developing from the fornix in rats. Phase contrast microcopy (bar = 100 microns).

inflammatory cells, and increased expression of pro-inflammatory cytokines such as IL-1β and TNFα in the conjunctiva (Marko et al., 2013; Ko et al., 2018). Spdef is expressed in the nuclei of human conjunctival GCs and its expression is reduced in patients with Sjögren syndrome. Spdef also plays a role in MUC5AC gene expression and in post-translational events leading to glycosylation and secretion (Chen et al., 2009).

GCs express receptors for growth factors including epidermal growth factor (EGF) and nerve growth factor (NGF) (Rios et al., 2007) and these growth factors seem to play an important role in GCs homeostasis. In conjunctival cell cultures, NGF can induce a dose-dependent increase of GC numbers, MUC5AC production, storage, and release (Lambiase et al., 2009), at least partially by promoting the differentiation of limbal epithelial cells into GCs in mice (Li et al., 2010). EGF also plays a critical role in the regulation of GC proliferation and mucin secretion, through the stimulation of the ERK1/2 pathway, translocating ERK1/2 to the nucleus and causing the proliferation of GCs (Shatos et al., 2008; Hodges et al., 2012).

Sex hormone regulation of mucin secretion has been suggested. In women with complete androgen insensitivity and polycystic ovary syndrome, androgens have demonstrated their involvement in the modulation of mucin production. Conjunctival samples of patients with complete androgen insensitivity syndrome showed an unchanged number of GCs, but a decrease of MUC1 and MUC5AC expression compared to controls (Mantelli et al., 2007). Women with polycystic ovary syndrome were shown to have increased conjunctival GCs and increased MUC5AC secretion than patients with polycystic ovary or healthy subjects (Bonini et al., 2007). However, in ovariectomized mice, hormone replacement therapy with estrogen and/or progesterone failed to show a modulation of MUC5AC and MUC4 in ocular surface epithelium (Lange et al., 2003).

2.3.3. Regulation of membrane mucin expression

MUC16 biosynthesis is post-transcriptionally regulated by Notch signaling at the early stage of epithelial cell differentiation in human conjunctival and corneal epithelial cells (Xiong et al., 2011). There is some evidence to suggest that not all membrane-bound mucins are controlled by the same mechanisms: a selective augmentation of MUC4 and MUC16, but not MUC1, was observed after retinoic acid or serum was added to the culture medium of a human conjunctival cell line (Hori et al., 2004). Another study showed that MUC1 and MUC16 gene expressions was upregulated while MUC4 gene expression was downregulated by dexamethasone in culture of human corneal epithelial cells (Seo et al., 2007). Others have shown that the eicosanoid 15-(S)-hydroxy-eicosatetraenoic acid (15S-HETE) is a selective secretagogue for MUC1 but not for MUC4 (Jumblatt et al., 2002).

3. How to investigate mucin deficiency?

Various diagnostic tools are recommended to investigate damage to the ocular surface and tear film instability such as the Schirmer test, TBUT, tear meniscus height, ocular surface staining, or tear osmolarity (Wolffsohn et al., 2017). Unfortunately, these tests are not very specific for mucin expression and GCs, and there is an unmet need for standardized methods to evaluate the state of conjunctival GCs and cell-associated mucins that could help to understand, diagnose and manage ocular surface diseases.

3.1. Tear ferning test

Tear ferning is a simple non-invasive test to assess mucus deficiency (Tabbara and Okumoto, 1982; Rolando, 1984; Vaikoussis et al., 1994). This test is based on the physical characteristics of mucus to crystallize and form ferns (arborisation) when dry at room temperature on a clean smooth surface and observed under microscope. The tear ferning pattern was proposed to be caused by the interaction of electrolytes, particularly sodium and chloride, with macromolecules such as tear film mucins and proteins (Pearce and Tomlinson, 2000). Thus, the result is not only an indicator of mucins, but also of tear osmolarity and quality of the tear film (Pearce and Tomlinson, 2000). A grading score of 4 patterns was previously proposed by Rolando on the basis of uniformity, branching, spreading and integrity of the mucus crystallization pattern (Rolando, 1984) (Fig. 3). It was reported that more than 80% of normal eyes had type I and type II patterns and more than 90% of DED patients had type III and type IV ferning patterns (Rolando, 1984). This ferning pattern was shown to be well correlated with tear production (Schirmer test) and tear film stability (fluorescein TBUT), but seems to be independent of individual tear proteins (Puderbach and Stolze, 1991). Its sensitivity and specificity were considered to be of the same order as the commonly used tests for Sjögren syndrome including Schirmer test, TBUT, rose bengal staining, or lactoferrin dosage in tears (Norn, 1994). However many ferning patterns do not easily fit into any of the Rolando grades described above, particularly around types I and II (Masmalli et al., 2014a). Thus, this test is currently not widely used (Masmalli et al., 2014b).

3.2. Ocular surface staining

Staining of the ocular surface is the most convenient and clinically feasible method for the evaluation of both the corneal and conjunctival epithelia integrity (Korb et al., 2008). The most frequent dyes used are sodium fluorescein, rose bengal, and lissamine green. The presence of a complete glycocalyx at the cellular apical surfaces, together with functioning tight junctions, is necessary to exclude dyes from the deeper cell layers (Bron et al., 2015).

Rose bengal, applied from a strip or as a solution, can be used to assess ocular surface barrier function. This staining method has been associated with reduced GC density and epithelial cell mucin expression in patients with aqueous tear deficiency (Pflugfelder et al., 1997). Staining is enhanced in human conjunctival cells with altered MUC16 extracellular domain glycosylation and it was suggested that rose bengal staining could be a result of either loss of expression or altered glycosylation of MUC16 (Blalock et al., 2008). However, rose bengal stains also healthy epithelial cells which are not protected by a normal mucin layer and as a consequence is not considered a vital dye. In addition, it has been demonstrated that the degree of staining is dose-dependent (Norn, 1973). Rose bengal is now infrequently used in clinical practice, because of significant discomfort and phototoxicity upon application, amplified when uptake is extensive (Bron et al., 2015).

Lissamine green is a nontoxic vital dye that is increasingly used to detect ocular surface damage. Lissamine green is considered an ideal dye for the detection of dead or degenerated cells (Tseng, 1994) and

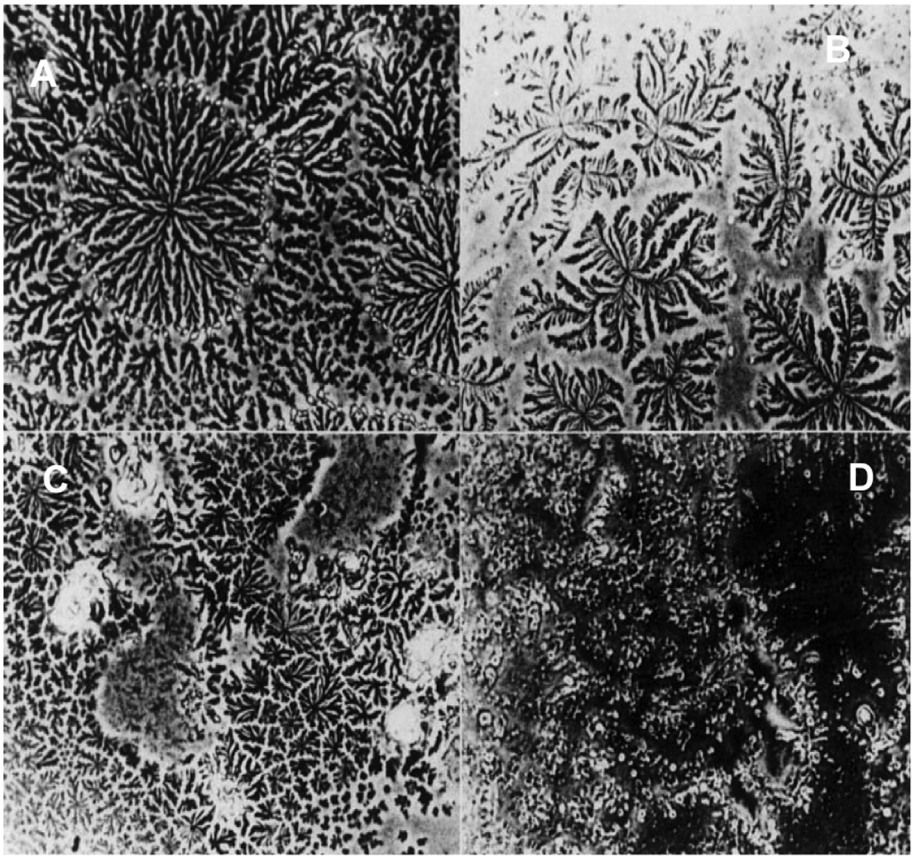

Fig. 3. Classification of ferning patterns. A: Type I: Note the uniform arborisation in all the fields of observation without spaces among the ferns. B: Type II. Empty spaces begin to appear among ferns. C: Type III. The single ferns are small and incompletely formed with rare or no branching. D: Type IV: The ferning phenomenon is absent. Magnification x 50. Adapted from Rolando et al. (1984).

unlike rose bengal, does not stain healthy epithelial cells nor affect their viability (Chodosh et al., 1994). Lissamine green stains epithelial cells only if the cell membrane is damaged, irrespective of the presence of mucins. Lissamine green may offer the same interpretations as staining with rose bengal on conjunctiva but not on cornea (Bron et al., 2015). The staining is enhanced if a red filter (Wratten No 25 equivalent) is used as a barrier filter on the slit lamp (Hamrah et al., 2011). In patients with mild to moderate DED, lissamine green 1% and rose bengal 1% showed a similar staining score using the van Bijsterveld scale (Machado et al., 2009). Both staining dyes show a low but significant association with symptom severity assessed with the ocular surface disease index (OSDI).

Fluorescein sodium is, among the three dyes, the most suitable for corneal staining while rose bengal and lissamine green are more adapted to the assessment of conjunctival damage. Fluorescein is water soluble and penetrates tissues where epithelial intercellular junctions are disrupted (i.e. cells that are dead or damaged). In patients with mild to moderate non-Sjögren syndrome aqueous deficient dry eye (NSS-ADDE), fluorescein staining was shown to correlate negatively with MUC16 in impression cytology samples (Gipson et al., 2011). A combination of fluorescein sodium and lissamine green is thus recommended to assess both the corneal and the conjunctival surface damage (Wolffsohn et al., 2017; Shiboski et al., 2017). The visibility of conjunctival fluorescein sodium staining can be greatly enhanced by the use of a yellow barrier filter (Wratten No 12 equivalent) (Peterson et al., 2006). In a recent study, conjunctival staining with fluorescein sodium and a yellow barrier filter was more sensitive in the detection of conjunctival damage than lissamine green (Eom et al., 2015). Another difference between dyes is that fluorescein sodium stains the less compromised cells whereas the lissamine green appears to stain the more compromised cells (Korb et al., 2008).

Fluorescein staining can be useful to identify conjunctival damage (sandbank epitheliopathy) shown as punctate staining spots over the conjunctiva due to prolonged and enhanced friction caused by lubrication deficiency. This specific conjunctival staining does vary with the severity of tear film insufficiency and thus may be a good indicator of enhanced friction between the inner surface of the lids and the surface of the conjunctiva (Van Setten, 2017). Staining of specific areas of the conjunctiva may also be characteristic of certain etiologies or mechanisms, such as mucus-fishing syndrome or drug toxicity if located in the nasal conjunctiva, or superior limbic keratoconjunctivitis if the staining is observed over the superior limbus and conjunctiva (Fig. 4).

3.3. Impression cytology

Impression cytology (IC) represents a practical and minimally

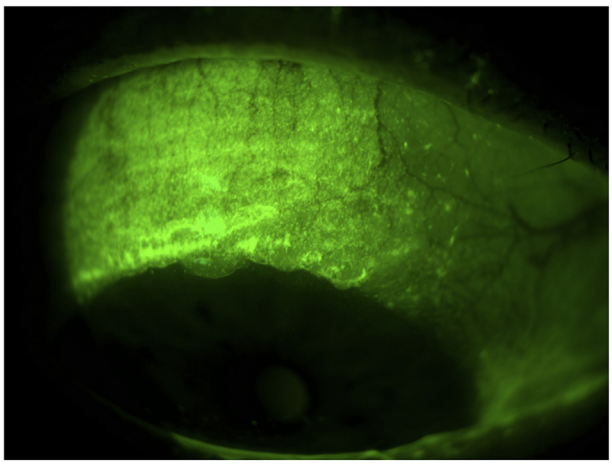

Fig. 4. Superior conjunctival fluorescein staining in a case of superior limbic keratoconjunctivitis.

invasive biopsy of the ocular surface epithelium performed under topical anesthesia with no side effect or contraindication. IC was first introduced in 1977 and is useful to assess damage caused by ocular surface disease including DED, ocular allergy, chronic conjunctivitis, or contact lens intolerance (Calonge et al., 2004; Hagan, 2017). This is an easy method to collect superficial cells including epithelial cells, mucin-secreting GCs and inflammatory cells infiltrating the conjunctiva. After topical anesthesia, strips of cellulose acetate or polyethersulfone filter papers are applied on the upper nasal and temporal bulbar conjunctiva, pressed gently and then removed (Baudouin et al., 1992). This involves removing one to three layers of conjunctival cells. The specimen is then fixed with ethanol or formaldehyde for histological analyses or can be processed for cellular or molecular biology techniques. Cells can thus be harvested from membranes and processed using various methods including light microscopy, transmission and scanning electron microscopy, immunofluorescence, immunocytochemistry, polymerase chain reaction, immunoblotting, or flow cytometry (Baudouin et al., 1997; Calonge et al., 2004). Traditionally, IC has been used to identify morphological changes at the ocular surface and to grade the squamous metaplasia (Singh et al., 2005). It was subsequently found to be useful for assessing biomarkers of potential ocular surface disease including inflammatory markers (HLA-DR, ICAM-1, metalloproteinases, cytokines, etc.), GC density, and mucins mRNA transcripts levels (Baudouin et al., 1997; Brignole et al., 2000; Shimazaki-Den et al., 2013, Corrales et al., 2011). Human GCs exhibited positive reactivity for alcian blue-periodic acid Schiff (PAS) reagent, goblet cell-specific cytokeratin-7 (CK7), HPA lectin, or MUC5AC (Fig. 5), but negative reactivity to the stratified squamous epithelial cell marker, cytokeratin-4 (Shatos et al., 2003). Using anti-CK7 antibodies, it is possible to determine the total number of GCs irrespective of their mucin content (Krenzer and Freddo, 1997). Thus, a decrease in CK7 staining indicates a loss of GCs (empty or filled) whilst an increase would reflect GC proliferation; a decrease in MUC5AC staining would indicate secretion, whereas an increase in MUC5AC staining would reflect inhibition of GC secretion.

Using impression cytology, Zhang et al. showed that symptoms of severity in DED were associated with decreased levels of MUC5AC and an overexpression of IL-6 (Zhang et al., 2013), and Corrales et al. demonstrated that MUC1 gene expression had the highest specificity and sensitivity for the diagnosis of DED, and thus proposed MUC-1 mRNA levels as a diagnostic marker of DED (Corrales et al., 2011). Accumulation of GCs over the cornea, interspersed or in place of the corneal epithelium, is common in patients with limbal stem cell deficiency (LSCD) (Fig. 6). Garcia et al. proposed to use IC as a novel diagnostic method for LSCD based on MUC5AC transcript detection in corneal epithelium by PCR-reverse dot blot (Garcia et al., 2013). The detection of the MUC5AC transcript in corneal epithelium was considered as a more sensitive method to diagnose LSCD than the conventional PAS-hematoxylin method (Garcia et al., 2012).

Conjunctival IC is currently not a first-line investigation in DED, but is often used in combination to diagnose and grade dry eye severity. In a case-control study, it was previously suggested that conjunctival IC was more specific, sensitive and had a higher predictive value for diagnosing dry eye than routine tear function tests like Schirmer test, TBUT, and rose bengal staining (Kumar et al., 2014). However, routine use of IC in clinical settings is limited by lack of facilities to stain and microscopically examine the filter paper. Another potential limitation is the lack of standardization regarding anesthesia, the type of filter paper, the area to be sampled, the pressure to be applied, and the operators skill to perform this investigation, as high interobserver variability was previously shown (Altinors et al., 2007; Doughty, 2016). In addition, although the technique is recognized as safe, frequent sampling in the same patient may lead to aggravation of an already damaged ocular surface (Rolando et al., 1994). In an effort to overcome these limitations, new devices (e.g. Eyeprim; Opia Technologies, Paris, France) have been designed for impression cytology and could be used for standardization. A pilot study showed that such a device yields similar amounts of RNAs and enough material for molecular analysis than conventional conjunctival impression cytology technics (Lopez-Miguel et al., 2017).

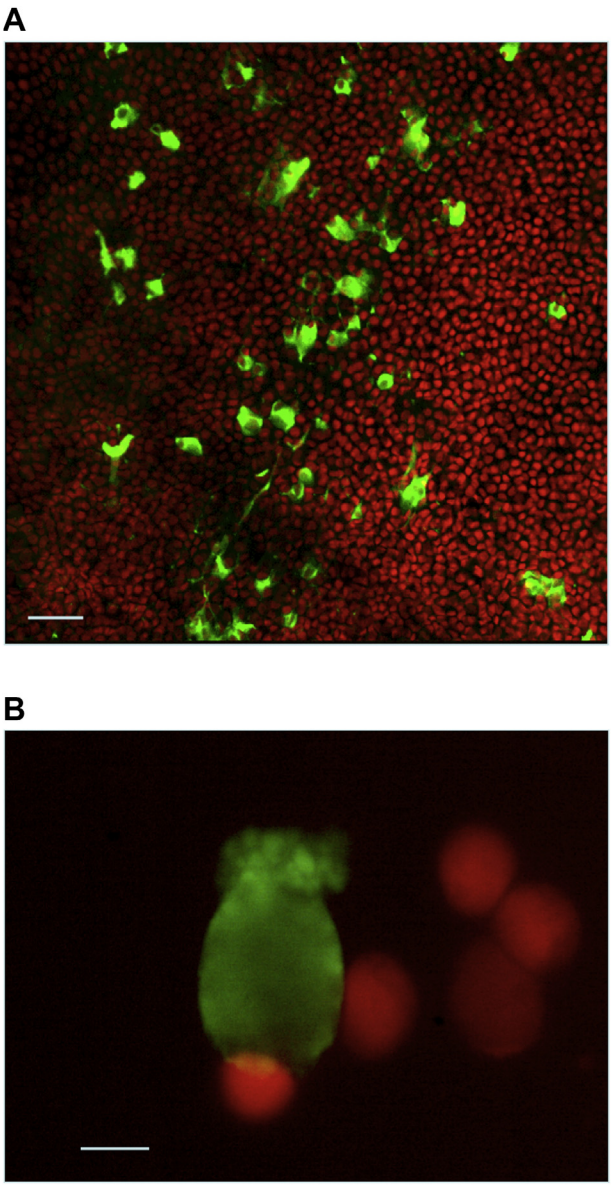

Fig. 5. Immunofluorescence staining of goblet cells in impression cytology. A and B: Confocal microscopy of a conjunctival impression cytology stained with anti-MUC5AC antibodies. B: MUC5AC staining in a goblet cell that appears with a polarized shape. Nuclei are stained in red with propidium iodide. A: bar = 100 microns; B: bar = 25 microns).

3.4. Laser scanning confocal microscopy

Laser scanning confocal microscopy (LSCM) or *in vivo* confocal microscopy (IVCM) of the conjunctiva and the cornea is an efficient and noninvasive supplementary diagnostic tool for the *in vivo* histological assessment of ocular surface diseases, providing a quantitative assessment of inflammatory cell density, epithelial cell size and density, as well as an evaluation of morphologic alterations of the ocular surface (Messmer et al., 2006). Several potential clinical applications have been proposed such as the assessment of DED severity, prognosis, differential diagnosis, and also helping with patient management and in the evaluation of responses to treatment (Villani et al., 2013a and Villani et al., 2013b for reviews). GCs can be visualized on confocal microscopic

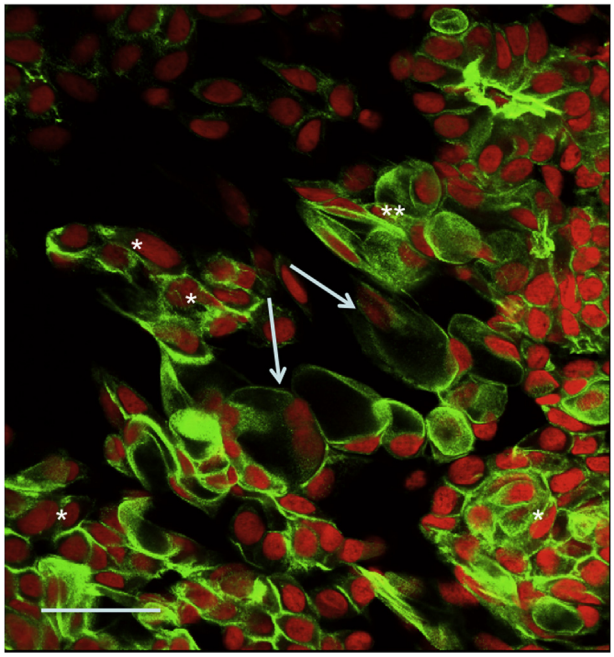

Fig. 6. Accumulation of goblet cells at the limbus in a case of limbal stem cell deficiency. Confocal microscopy in impression cytology using MUC5AC immunostaining. Nuclei are stained in red with propidium iodide. Note that some GCs are empty of MUC5AC-stained products (arrows). Most of MU5AC-positive cells are small, with centered nuclei and are likely immature GCs (*), some are typical GCs with their positive cytoplasm and decentered nucleus (**) (bar = 100 microns).

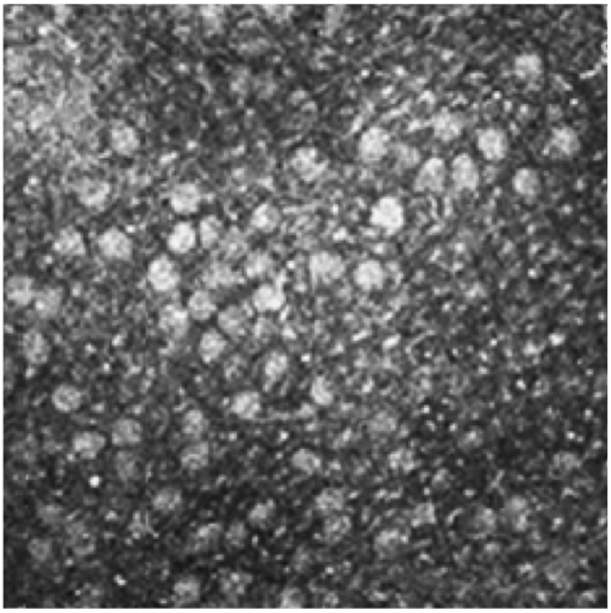

Fig. 7. In vivo confocal microscopy of goblet cells (frame size = 400 × 400μ). All large white round cells are likely goblet cells.

images (Fig. 7). They appear as large to giant hyperreflective round to oval shape cells with a nucleus displaced peripherally or as a central pore, sometimes crowded in groups throughout the epithelium (Messmer et al., 2006). Patterns may differ according to the contrast with the surrounding tissues and hyporeflective structures can also occasionally be observed, appearing as intraepithelial microcysts. There exact nature is not yet determined but they could correspond to empty GCs or GCs with contents other than mucins. More recent investigations in healthy subjects and in Sjögren syndrome aqueous deficient dry eye (SS-ADDE) patients confirmed that GCs can be monitored with IVCM (Colorado et al., 2016a; Hong et al., 2010). GC density determined with LSCM can be used for the diagnosis of conjunctivalization associated with LSCD (Lee et al., 2010) and as a prognostic factor in patients undergoing glaucoma filtering surgery (Di Staso et al., 2018). Beside the initial cost of the machine, one other important limitation of IVCM is the time and the training required to acquire conjunctival and corneal images.

3.5. Measurement of tear film instability

The mechanisms of normal tear film breakup involve tear film thinning via evaporation, subsequent transient lipid contamination, and local decrease of wettability over the glycocalyx (Sweeney et al., 2013). Instability of the tear film and related tear film breakup is traditionally evaluated via fluorescein breakup time (FBUT) and via non-invasive methods such as interferometry and topography (Wolffsohn et al., 2017). Tear film instability is measured by observation of the first dark areas which appear in the fluorescent tear film. Recent investigations suggest that fluorescein break-up pattern (area; spot, line; dimple; random) could discriminate between the different mechanisms of DED. In particular, a spot-like pattern would be suggestive of poor wettability of the cornea, such as in mucin abnormalities, and a dimple pattern would suggest an additional mechanism of drag and suction of tears by eyelids edges (Yokoi et al., 2017).

4. Mucins and inflammation: what is the relationship ?

Over the last few years, research has advanced our understanding of the complex relationship between ocular surface inflammation and mucin expression by GCs and epithelial cells. There is experimental evidence that inflammatory mediators act directly on GCs (and other cells of the ocular surface) to modulate proliferation, differentiation, apoptosis and function (Conteras-Ruiz et al., 2013). More recent investigations suggest a role for GCs in immune homeostasis which may be dysregulated in ocular surface diseases.

4.1. Mucins and ocular surface inflammation

Both innate and adaptive immune cells appear to produce cytokines which can directly regulate GC proliferation, apoptosis and mucin secretion. In mouse dry eye models, various pro-inflammatory cytokines (e.g. TNFα, IL1β, IL-6, IL-8) are produced when the corneal and conjunctival epithelia are injured. These pro-inflammatory cytokines are produced by resident γ/δ T cells and by epithelial cells themselves, and activate resident antigen-presenting cells (APCs), mainly dendritic cells, initiating the adaptive immune response and the infiltration of Th1 and Th17 cells in the conjunctival and lacrimal gland. In response to inflammatory stimulation, GCs and corneal/conjunctival epithelial cells express receptors for inflammatory cytokines such as IFN-γ, TNFα, IL-6, IL-17A and IL-13 (Dartt and Masli, 2014). Among these cytokines, IFN-γ (a Th1 cytokine) plays a central role in inducing conjunctival metaplasia and decreasing the number of filled GCs (De Paiva et al., 2007). Desiccating stress in mice produces increased expression of IFN-γ whereas IL-13 (a Th-2 cytokine, also produced by NK/NKT cells) transcripts are downregulated and IFN-γ is believed to antagonize the protective effect of IL-13 on conjunctival GCs (Pflugfelder et al., 2013). In primary cultures of mouse GCs, IFN-γ and TNFα were shown to inhibit mucin secretion induced by cholinergic stimulus and lead to GC apoptosis and death (Contreras-Ruiz et al., 2013; Dartt and Masli, 2014). Conversely, IFN-γ neutralization prevented conjunctival GC loss in an experimental murine dry eye model (Zhang et al., 2014). It has also been suggested that the constitutive expression of IL-13 may be required for homeostatic control of GCs (De Paiva et al., 2011) since conjunctival GC density is markedly reduced in IL-13 knock-out (KO)

mice (De Paiva et al., 2011). IL-13 could stimulate conjunctival GCs proliferation without affecting mucin secretion (Contreras-Ruiz et al., 2013). Others have demonstrated that in conjunctival epithelial cell cultures, IL-13 stimulates MUC5AC and MUC2 glycoprotein production (Tukler Henriksson et al., 2015). IL-13 could also induce the transcriptional factor Spdef which was reported to be of particular importance in epithelial differentiation and GC formation (Gipson, 2016).

Furthermore, De Paiva et al. also reported that desiccating stress upregulates the Th-17 pathway, and thus IL-17 could play a major role in acute corneal barrier dysfunction, possibly by the up-regulation of metalloproteinases (De Paiva et al., 2009). However, in other experiments, IL-17 was suggested to represent a mechanism that replenishes the loss of GCs in the conjunctiva. Both IL-6 and IL-17 can induce proliferation of conjunctival GCs, but only IL-6 enhances mucin secretion by cholinergic stimulus (Contreras-Ruiz et al., 2013).

The effect of ocular surface inflammation has been also investigated in relation to membrane-bound mucins and it was suggested that the loss of membrane-bound mucins and/or their ectodomain release induced by pro-inflammatory cytokines may potentially cause loss of tear film stability and rapid tear break-up (Albertsmeyer et al., 2010). Paulsen et al. showed that pro-inflammatory cytokines including IL-1α and IL-1β, but not IFN-γ or TNFα, downregulate expression and production of MUC16 in human corneal cells. In addition, IFN-γ or TNFα increased shedding of membrane-bound MUC16 from cultured corneal epithelial cells. They concluded that downregulation of MUC16 could be a mechanism that contributes to deterioration in DED (Paulsen et al., 2008). In another study using a human corneal limbal epithelial cell line, IL-6 downregulated MUC1 expression while IFN-γ and TNFα upregulated MUC1 and MUC16 and induced MUC1 and MUC16 ectodomain proteolysis (Albertsmeyer et al., 2010). Upregulation of MUC1 may be a compensatory response to inflammation.

4.2. Mucins and neurogenic inflammation

The ocular surface is extensively supplied by sensory and autonomic nerve fibers that play a crucial role in maintaining ocular surface homeostasis (Belmonte et al., 2017). Chronic ocular inflammation leads to a decrease in corneal sensitivity and a consequent neurosecretory block that reduces reflex tear secretion (Lambiase et al., 2011). Since GC secretion is stimulated via a reflex arc from the afferent sensory nerves in the cornea to the efferent sympathetic and/or parasympathetic nerves of the conjunctiva, the loss of nerve function may alter GC secretion (Dartt and Masli, 2014).

Neurogenic inflammation is produced principally through release of neuromodulators, such as substance P and calcitonin-gene related peptide (CGRP), which leads to a breakdown of the blood-tissue barrier, edema, and release of polymorphonuclear leukocytes into the tears (Beuerman and Stern, 2005).

Nerve growth factor (NGF) and its receptors are highly expressed on the ocular surface and may play a role in several ocular surface diseases, including dry eye (Lambiase et al., 2011). Both human conjunctival and corneal cells (epithelial, stromal and endothelial cells) have the ability to produce and release NGF, and to express its receptors. It has been suggested that NGF was a main player in the neuroimmune cross-talk of the ocular surface as well as in the stimulation of corneal sensitivity, epithelial proliferation and differentiation, and stimulation of mucin production by GCs (Mantelli et al., 2013a). Studies of conjunctival GCs have shown that NGF can stimulate mucin secretion (Ríos et al., 2007), and topical application of NGF can increase the number of GCs in dogs with dry eye (Coassin et al., 2005). In a rat model, electrical stimulation of the trigeminal ganglion can result in a significant decrease of GC density, and substance P, released from sensory nerve endings, could influence GC function (Kovacs et al., 2005). Thus, substance P and calcitonin gene-related peptide (CGRP) released from the corneal and conjunctival sensory nerve endings interact directly with the GCs to stimulate MUC5AC secretion (Kovacs et al., 2005).

4.3. Mucins and immune tolerance

As part of the immune surveillance system, innate immune cells induce an acute inflammatory response to clear offending agents and activate local antigen-presenting cells to initiate an adaptive immune response in local draining lymph nodes (Bron et al., 2014). The ocular surface can modulate the immunological response in order to avoid possible negative consequences of an exaggerated response or chronic activation of the immune system. It seems that both membrane-bound mucins, and GCs secreted mucins play an important immunomodulatory function at the ocular surface.

Corneal and conjunctival epithelial cells including GCs express multiple toll-like receptors (TLR) whose activation plays a crucial role in the early response against pathogens and foreign- or self-antigens (Kojima et al., 2008; McGilligan et al., 2013). In human corneal epithelial cells in culture, MUC1 and MUC16 modulate the inflammatory response by limiting the TLR2-and TLR5-induced expression of proinflammatory cytokines including IL-6, IL-8 and TNFα (Menon et al., 2015), suggesting that these transmembrane mucins may contribute to the maintenance of immune homeostasis by limiting the TLR-mediated innate immune response. However, MUC16-deficient mice display spontaneous subclinical conjunctival inflammation (Shirai et al., 2014).

Several recent studies also suggest that the loss of GCs may contribute directly to a loss of immune homeostasis favoring chronic inflammation (Barbosa et al., 2017; Contreras-Ruiz and Masli, 2015; Ko et al., 2018). It was demonstrated that the loss of conjunctival GCs in Spdef-KO mice reduces the tolerance-inducing properties of antigen presenting cells (APCs) in the conjunctiva and draining cervical nodes and thus abrogate immune tolerance to antigens at the ocular surface (Ko et al., 2018). Such immune tolerance seems to be mediated by GC antigen passage (GAP) in the conjunctiva which allows soluble antigens to pass from the apical to basolateral membrane closed to dendritic cells as demonstrated in intestinal mucosa (McGuckin and Hasnain, 2017). GAP opening may be mediated by cholinergic stimulation and mucin secretion and be abolished by GC loss such as in DED (Barbosa et al., 2017). In the same way, an increased number of dendritic cells expressing IL-12 (a Th1 cytokine) was observed in Spdef KO mice and consequently it was suggested that GCs could suppress IL-12 expression in dendritic cells (Barbosa et al., 2017).

TGF-β2 is one of the most important mediators of immune privilege, acting as a soluble immunomodulatory factor that suppresses cells and molecules that mediate innate and adaptive immune inflammation (Hori et al., 2010; Stern et al., 2010). It was shown that mouse conjunctival GCs express and activate TGF-β2 in response to a TLR4-mediated stimulus in cultures. Since TGF-β2 produced by GCs can also modulate dendritic cell phenotype towards an immature or a tolerogenic type (Pfugfelder et al., 2008), this provides an immunomodulatory cross-talk between conjunctival GCs and dendritic cells (Contreras-Ruiz and Masli, 2015). Interestingly, GC restoration in dry eye patients treated with topical cyclosporine A (CsA) has been associated with a concomitant increase in TGF-β2 expression in the bulbar conjunctiva (Kunert et al., 2002; Pflugfelder et al., 2008).

In DED, the chronicity of the disease suggests that dysregulation of immune mechanisms leads to a circle of continued inflammation, accompanied by alterations in both the innate and adaptive immune responses (Wei and Asbell, 2014). The implication of GC and mucin dysfunction in adaptive immunity leads to a revisit of the DED vicious circle and propose goblet cell dysfunction as a possible entry into this model (Fig. 8).

In DED, mucin alteration leads to tear film instability and elevation of tear osmotic pressure, activating an inflammatory reaction and causing ocular surface damage, epithelial cell apoptosis, and a decrease in GC and mucin functions, perpetuating the vicious circle of DED (Baudouin et al., 2018). Inflammatory cytokines appear to play a

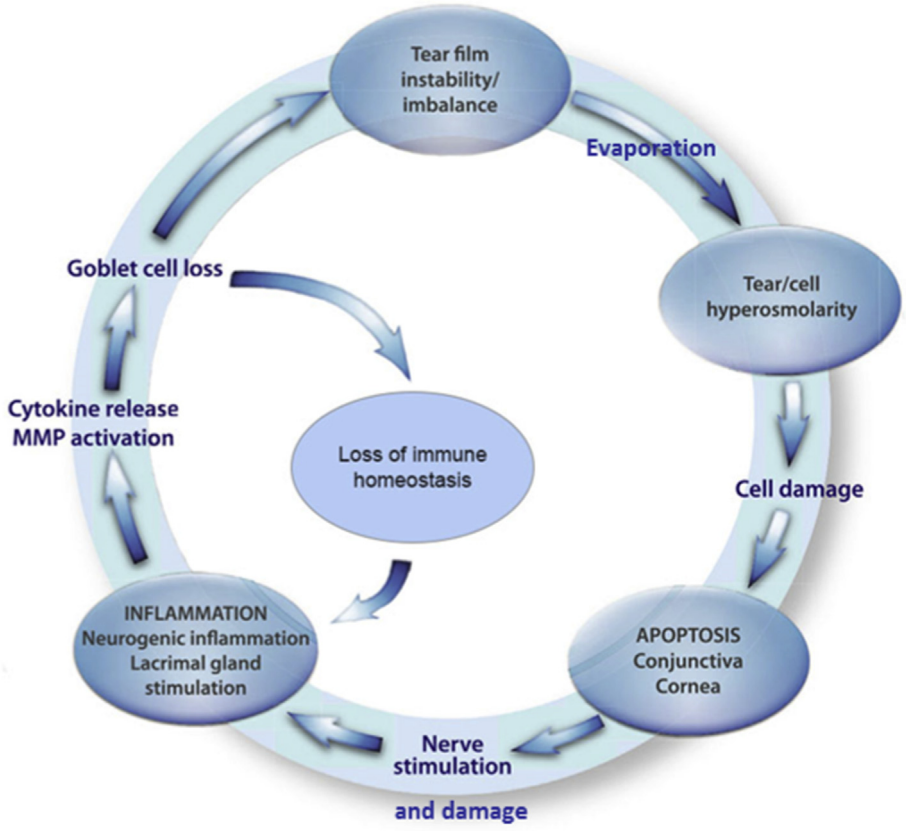

Fig. 8. Revisited vicious circle hypothesis (adapted from Baudouin et al., 2013).

central role in the initiation and propagation of the inflammatory reaction triggered by increased osmotic pressure. Destruction and/or dysfunction of GCs may cause a loss of immunosuppressive properties driven by mucin-secreting cells, and further enhance chronic inflammation by loss of their negative feedback to dendritic cells (Contreras-Ruiz and Masli, 2015).

5. Relations between mucins and ocular inflammatory surface diseases

Alongside results from animal models suggesting the role of membrane or secreted mucins in the pathology of dry eye or allergic ocular disease, human data has shown different possible associations between acute or chronic inflammation and according to the stage of the disease. In early stage of ocular disease, GC proliferation and mucin secretion can be increased as a protective response, but later in the disease process, GC mucin secretion and membrane-bound mucin expression can be decreased leading to more severe ocular surface pathology as part of the vicious circle.

5.1. Dry eye disease

DED is a common disease with various etiologies and severity presentation (Craig et al., 2017). Ocular inflammation and GC loss and reduced levels of MUC5AC have been described in every form of dry eye including SS-ADDE, NSS-ADDE and evaporative dry eye (EDE) (Argüeso et al., 2002). In patients with DED, the percentage of conjunctival GCs has been negatively correlated with up-regulation of HLA-DR and ICAM-1 expression (Pisella et al., 2000), suggesting that GC depletion may be correlated with the severity of the inflammatory reaction and subsequent detrimental effects on the ocular surface. It was also suggested that altered mucin glycoproteins on the surface of apical conjunctival cells rather than protein level may occur as the direct result of inflammation (Danjo et al., 1998; Gipson, 2004; Stephens and McNamara, 2015).

In a recent study of mucins, inflammatory markers, and clinical tests in dry eye patients, Zhang et al. showed that MUC5AC expression in IC samples was negatively correlated with the OSDI and weakly but significantly positively correlated with TBUT (Zhang et al., 2013). Pflugfelder et al. found that GCs (including both filled and empty cells) density was markedly reduced in SS-ADDE and NSS-ADDE but not in EDE patients (mainly MGD) compared to control subjects (Pflugfelder et al., 2015). Overall, they found a negative correlation between the IFN-γ mRNA and disease severity and GC density in the temporal bulbar conjunctiva.

However, there is still a lack of consistency regarding the role of membrane-bound mucins in the pathophysiology of DED. An O-acetyl sialic acid component of MUC16 was shown to be altered on the apical cell surface of NSS-ADDE patients, suggesting that an alteration of mucin O-glycosylation in dry eye compromises the ocular surface epithelial barrier making it more susceptible to epithelial damage (Sumiyoshi et al., 2008). This is consistent with early investigations showing that the binding of an antibody to a carbohydrate epitope (later identified as a MUC16 epitope) (Argüeso et al., 2003) to conjunctival cells of NSS-ADDE patients was reduced with significant correlation to disease severity as assessed by rose bengal staining (Danjo et al., 1998). In patients with SS-ADDE, Argüeso et al. found no significant change in mRNA expression of MUC1 and MUC4, while conjunctival MUC5AC mRNA transcript and MUC5AC protein in tears were reduced compared with normal individuals (Argüeso et al., 2002). Similarly, Caffery et al. generally found no difference in MUC1 and MUC16 expression between NSS-ADDE patients and controls, but higher MUC1 and MUC16 mRNA levels were found in patients with SS-ADDE compared with NSS-ADDE patients and the control group (Caffery et al., 2008, 2010). By contrast, Corrales et al. found that the mRNA expressions of MUC1 and MUC4 (together with MUC5AC and

MUC2) in conjunctival epithelium were significantly lower in patients with moderate to severe NSS-ADDE compared with healthy subjects (Corrales et al., 2011).

In postmenopausal women, symptomatic for mild-to-moderate NSS-ADDE, Gipson et al. found an increased expression of mRNA and the corresponding membrane-bound MUC1 and MUC16 from IC samples. These changes correlated positively with several diagnostic tests including conjunctival and corneal staining, and dry eye symptom severity score. By comparison, MUC5AC in tear samples was not significantly different between symptomatic patients and controls, but there was a trend for increased cellular MUC5AC (Gipson et al., 2011). It was suggested, that the upregulation in expression of membrane mucins may be a compensatory response to repair local areas of mucin loss on apical surfaces due to ectodomain release potentially induced by inflammatory mediators (Gipson et al., 2011). In another study with post-menopausal women with NSS-ADDE, increased symptom severity significantly correlated with increased MUC16 expression but lower MUC16 protein in tear film (Srinivasan et al., 2013). As reported by Gipson et al., it is possible that changes in GC number and thus MUC5AC secretion do not occur until later stages of disease. This is consistent with a previous study showing that mucin level could change according to the stage of the disease, possibly with an initial increase of conjunctival GCs followed by a decrease in the chronic stage of the disease (Pisella et al., 2000).

5.2. Ocular allergy

Ocular allergy includes distinct clinical conditions such as seasonal or perennial allergic conjunctivitis, vernal keratoconjunctivitis (VKC), and atopic keratoconjunctivitis (AKC) (Leonardi, 2002). Conjunctival GCs are a direct target of cytokines and chemokines produced during the allergic reaction and respond by increasing production of MUC5AC that functions to remove allergens from the tear film and protect the ocular surface (Dartt and Masli, 2014). In addition to neural reflex stimulation, MUC5AC secretion is enhanced by histamine, leukotrienes and prostaglandins generated by activated mast cells and eosinophils (Dartt et al., 2011; Dartt and Masli, 2014).

VKC is a persistent form of ocular allergy characterized by severe inflammation frequently associated with corneal complications and the formation of giant papillae (Leonardi et al., 2012). The immune reaction is characterized by the presence of an increased number of Th2 lymphocytes, eosinophils, activated mast cells and fibroblast infiltration (Leonardi, 2002). Patients with VKC show increased density of conjunctival GCs and increased levels of MUC5AC, suggesting a protective mechanism aimed at clearing allergens from the ocular surface. In VKC the two main features of GCs can be distinguished using impression cytology: 1) increased number of GCs and mucus strand compared to controlled subjects and, inversely, 2) reduced number of GCs associated with keratinization and squamous metaplasia and a higher degree of damage, as shown by weaker intercellular junctions (Aragona et al., 1996).

AKC is a persistent inflammatory, bilateral condition involving the eyelids, the conjunctiva, and possibly the cornea and can be defined as the ocular manifestation of atopic dermatitis (Leonardi et al., 2012). In patients with severe AKC, MUC1, MUC4, and MUC16 mRNA expression is significantly upregulated with significant downregulation of MUC5AC mRNA expression compared with control eyes (Dogru et al., 2006, 2008). Compared to patients with VKC, the conjunctival GC density is reduced in patients with AKC, while the expression of MUC1, MUC2 and MUC4 was significantly increased (Hu et al., 2007). It has been proposed that increased expression of transmembrane mucin may represent a defense mechanism to compensate for the loss of MUC5AC in these patients (Mantelli et al., 2013a).

5.3. Filamentary keratitis

Filamentary keratitis is a chronic, recurrent, and debilitating condition observed in various ocular pathologies in the presence of severe aqueous deficiency (Albietz et al., 2003; Tabery, 2003; Tanioka et al., 2009). This is associated with basal epithelial cells and basal membrane degeneration due to desiccation and increased friction during blinking. The *in vivo* morphology is consistent with aggregation of mucus and cell debris adhering to the corneal surface (Tabery, 2003). Mucins (MUC5AC and MUC16) firmly adhering to the corneal epithelial may initiate the filament formation (Tanioka et al., 2009).

5.4. Superior limbic keratoconjunctivitis

Superior limbic keratoconjunctivitis (SLK) is a disease characterized by inflammation of the upper palpebral and superior bulbar conjunctiva, keratinization of the superior limbus and corneal and conjunctival filaments. It may be associated with other diseases such as DED, hyperthyroidism and hyperparathyroidism. SLK is typically caused by local mucin deficiency in the upper conjunctiva, resulting in abnormal friction and inflammation between the upper eyelid and the superior conjunctiva and corneal limbus (Nelson, 1989).

5.5. Mucus fishing syndrome

Mucus fishing syndrome is characterized by persistence of ocular symptoms such as irritation associated with excessive mucus accumulation at the ocular surface or the inferior cul-de-sac in patients with external ocular disease including DED, blepharitis, or ocular allergy. The mucus accumulation often presents as a stringy discharge that the patient tends to remove. Mechanical or digital removal of the excess of mucus can create persistent ocular surface irritation and inflammation leading to further increase in mucus production and excessive mucous discharge (McCulley et al., 1985; Slagle et al., 2001).

5.6. Contact lens wear

Contact lens (CL) wear may cause mechanical friction which can result in superficial corneal epithelial cells damages and an ocular surface inflammatory response (Efron et al., 2013). Whether the GCs and mucin secretion is altered in CL wear has not been clearly established because of various factors including lens-related factors such as material (e.g. conventional hydrogels versus silicone hydrogels), replacement frequency (e.g. daily/monthly replacement), duration of CL wear and methodological factors such as impression cytology (Colorado et al., 2016a; Hori et al., 2006; Corrales et al., 2009; Doughty, 2011). A recent study using IC and IVCM, showed that CL wear induced a reduction of GC density over 6 months and this reduction was exacerbated in CL wearers with dry eye symptoms (Colorado et al., 2016b). A previous study also showed reduction in MUC5AC production in both rigid permeable and soft CL wearers (Pisella et al., 2001). Consistently, ocular surface analysis using a fluorescein-label lectin suggests a reduction and/or compositional alteration of glycocalyx in soft contact lens wearers, which was significantly correlated with reduced TBUT (Fukui et al., 2016).

Over the long-term ($\geq$5 years) in tolerant CL wearers, neither mucin mRNA expression by conjunctival epithelia nor mucin content per unit protein in tears was altered compared to non-CL wearers (Hori et al., 2006). Mucin expression appeared to be upregulated during the early years of contact lens use, but returned to normal levels with long-term lens wear (Ramamoorthy and Nichols, 2008).

Dry eye symptoms in CL wearers is frequently associated with epitheliopathy (Korb et al., 2002) due to inadequate lubrication between the lid wiper surface and the ocular surface, resulting in physical trauma and damage to the lid wiper and, to a lesser degree, the other components of the ocular surface. Indeed, lid wiper epitheliopathy

(LWE) and lid parallel conjunctival folds (LIPCOF) in CL wearers with dry eye symptoms are associated with a decreased mucin concentration in ocular surface fluids and mucins adhering to contact lens. It was suggested that increased friction may result from insufficient mucins, or an altered composition of the resident mucins at the ocular surface (Berry et al., 2008).

5.7. Benzalkonium chloride toxicity

Ocular surface disease including DED is common among patients with glaucoma and this has been correlated with the exposure to benzalkonium chloride (BAK) in antiglaucoma eye drops (Baudouin et al., 2010). In a rat model, BAK at 0.25% and 0.5% appeared to cause a loss of GCs, associated with increased corneal thickness, apoptosis, corneal inflammation, and neovascularization (Pauly et al., 2007). Even low concentrations of BAK can induce GC loss and increase the cytoplasmic/nucleus ratio, two characteristics observed in patients with DED (Rolando et al., 1991). In human corneal-limbal epithelial cell culture, membrane-bound MUC1 and MUC16 are also reduced by short-term exposure to commercial eye drops preserved with BAK (Chung et al., 2006). Short-term exposure of antiglaucoma medication containing 0.01% BAK caused rapid damage to the ocular surface, most notably the mucus layer of the tear film (Herreras et al., 1992). In another study, patients who received timolol eye drops preserved with BAK at 0.01% and 0.04% had lower Schirmer test scores, shorter TBUTs, reduced GC densities, and a greater amount of epithelial cell squamous metaplasia when compared with healthy patients (Yalvacs et al., 1995). Patients treated over the long-term with preserved latanoprost or preserved timolol eye drops showed significant subclinical inflammation associated with a reduction of MUC5AC positive cells compared with normal eyes or patients treated with preservative-free drugs (Pisella et al., 2004) (Fig. 9). The authors also noted that latanoprost-treated eyes exhibited higher MUC5AC-positive cells than timolol-treated ones, despite higher or similar BAK exposure, suggesting the possible effect of prostaglandin analogs on GCs (Pisella et al., 2004). In another study, patients with open angle glaucoma or ocular hypertension showed a significant reduction in GC density as assessed by IVCM after 6 months of treatment with preserved levobunolol (Ciancaglini et al., 2008). Recently, correlation analyses showed a strong negative association between GCs density and TBUT, and with OSDI score in patients treated with preserved antiglaucoma medications, suggesting that reduction of GC density may play a pivotal role in the pathophysiology of glaucoma-related ocular surface diseases (Di Staso et al., 2018).

5.8. Glaucoma filtering surgery

Glaucoma filtering surgery is a procedure in which an intra-scleral fistula drains the aqueous humor from the anterior chamber into the subconjunctival space (filtration bleb). Functioning blebs are associated with a higher number of intraepithelial microcysts (Meziani et al., 2016) and the absence of GCs and/or microcysts is a factor of poor prognosis after filtering surgery (Baudouin, 2013). Amar et al. demonstrated the presence of numerous GCs, but empty of soluble MUC5AC, at the surface of the functioning blebs contrasting with highly and homogeneously stained GCs outside the limit of the blebs (Amar et al., 2008). These cells appeared to correspond to the microcysts observed at the surface of functioning blebs (Fig. 10). By contrast, non-functioning blebs showed very low number of GCs/microcysts. These empty GCs are filled with aqueous humor and may play a role in the drainage of the aqueous humor through the bleb wall. The outcome of trabeculectomy has been negatively correlated with pre-operative GC density and positivity for MUC5AC and it was suggested to consider GCs density as an indicator of glaucoma filtration surgery outcome (Agnifili et al., 2016; Matropasqua et al., 2017). It is not known if empty GCs in functioning blebs are able to produce and release TGFβ2 whose anti-inflammatory activity could explain the outcome of the surgical filtration. However, as recently reported, attrition, as part of the additional mechanical stress on the conjunctiva after glaucoma surgery associated bleb creation (Van Setten, 2018) could contribute to the loss of GCs. The basic mechanism could be similar to that reported for conjunctival changes after contact lens wear (Doughty, 2011).

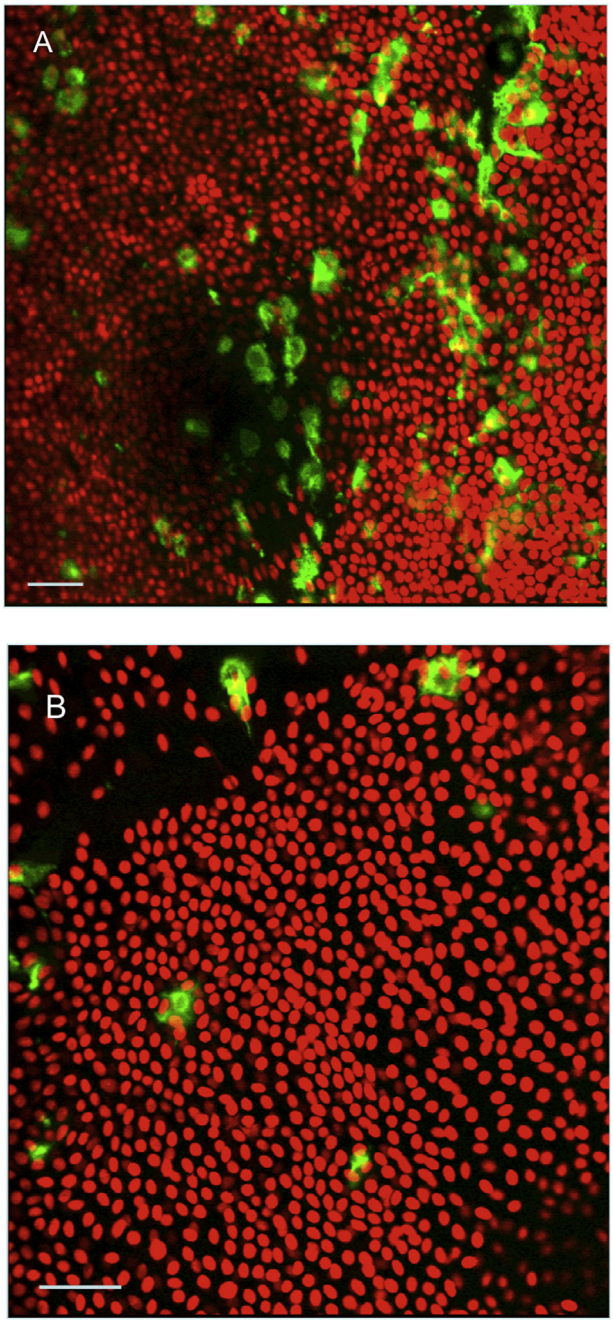

Fig. 9. Decreased density of GCs in glaucomatous patients. Confocal microscopy of impression cytology specimens and immunostaining of MUC5AC. Nuclei are stained in red with propidium iodide. A: normal density; B: dry eye induced by antiglaucoma eyedrops (bars = 100 microns).

5.9. Mucins and ocular surface infection

Ocular surface mucins play a rather important role protecting the corneal and conjunctival epithelia. Both gel-forming and cell surface-associated mucins contribute to the formation of a protective system against potential infection at the ocular surface. Gel-forming mucins have the capability to lubricate the ocular surface but also can capture

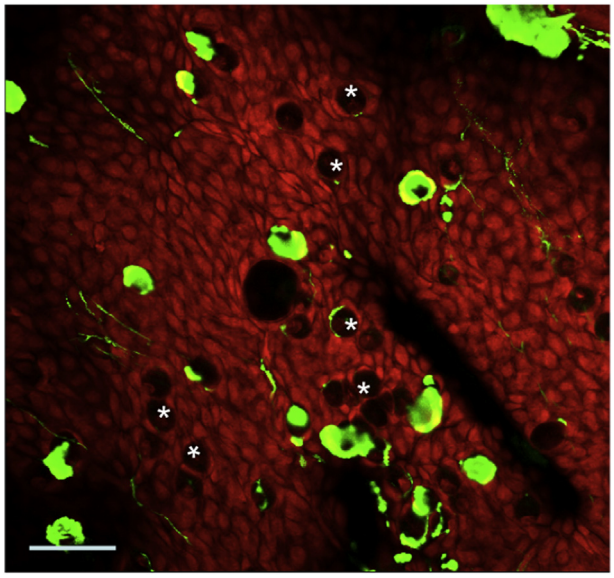

Fig. 10. Microcysts at the surface of a functioning filtering surgery bleb. Confocal microcopy in impression cytology after MUC5AC immunostaining. Both positive and negative cells are visible. The negative cells (*), with elongated nuclei are most likely empty GCs based on their shape, size very similar to the MUC5AC positive cells. They are empty of immunostaining, likely due to transepithelial passage of aqueous humor from the subconjunctival space (bar = 100 microns).

pathogens to facilitate their clearing from the ocular surface. Cell surface-associated mucins reduce abrasive stress and could form an apical cell surface barrier protecting against pathogens.

Therefore, disruptions of mucus production, composition, or clearance may be an important factor in the potentially enhanced susceptibility to ocular surface infection, including the well-known risk of infectious keratitis in contact lens wearers and a paradoxically much less frequent risk in patients with dry-eye conditions (Narayanan et al., 2013).

On the other hand, *Chlamydia trachomatis* infection has been associated with a reduced expression of several mucins (MUC1, MUC4, MUC5AC, MUC7) (Ramadhani et al., 2017). Animal experiments in rabbits and rats have shown that ocular mucins inhibit the adherence of infectious agents, e.g. *Pseudomonas aeruginosa*, to uninjured corneal epithelium (Feiszlig et al., 1994). Inversely, several pathogens can develop virulence mechanisms to modify the mucin structure. As examples, some strains of *Pseudomonas aeruginosa* express a protease which is able to cleave disulfide bonds of mucins (Aristoteli and Willcox, 2003), while some other strains also express glycosidase activity leading to further degradation of the mucin carbohydrate chains. This eventually weakens the protective mucosal coat and facilitates bacterial growth and penetration of the underlying epithelial cells (Aristoteli and Willcox, 2003). Another example comes from a bacterial metalloproteinase (ZmpC) secreted by *Steptococcus pneumoniae* (a non-opportunistic bacteria): this enzyme induces ectodomain release of MUC16 from epithelial cell surface, suggesting a mechanism that promotes the loss of the membrane mucin barrier function and the onset of infections (Govindarajan et al., 2012). Additionally, as MUC16 contributes to the maintenance of immune homeostasis (Menon et al., 2015), the release of MUC16 ectodomain may potentially promote an inflammatory response to clear the invading non opportunistic bacteria (Govindarajan et al., 2012). A similar mechanism of mucin network destruction has been proposed for viral adenovirus keratoconjunctivitis (Menon et al., 2016). Interestingly, epithelial cells of injured corneas express higher levels of galectin 3 (Cao et al., 2002), and this imbalance between mucins and galectin 3 likely promotes HSV1 adhesion (Woodward et al., 2013). On the other side, Herpes simplex type 1, but not type 2, may use human galectin 3 as a co-entry factor to penetrate into epithelial corneal cells, while the transmembrane mucins, in normal conditions, counteract this pro-infection mechanism by binding galectin 3 (Woodward et al., 2013; King et al., 2009). Finally, the soluble mucin MUC7 has been shown to have an antifungal and antimicrobial activity in the salivary gland (Bobek and Situ, 2003). Although MUC7 has not been found in tears, it may also have antimicrobial properties in the ocular surface, including the lacrimal gland.

6. Treatments to restore mucin functions

As comprehensively described in this review, mucin dysfunction, including loss of GC-secreted mucins, is common in all forms of at least moderate to severe DED and related disorders. Treatments which are able to protect GCs and to restore mucin functions may thus be powerful tools in the armamentarium of therapeutic strategies targeting the ocular surface homeostasis. Different options have been proposed including mucinomimetics, secretagogues, anti-inflammatory agents, or growth factors. Mucolytics can also be proposed to decrease mucus discharge and accumulation in some other ocular pathologies. In all cases, these treatments should be ideally formulated without a deleterious preservative to avoid adverse effect on membrane-bound and secreted mucins.

6.1. Mucomimetics

Several gel lubricant eye drops containing polymers are claimed to be “mucin-like” including hyaluronic acid, carbomers and HP-Guar. They have been designed to prevent surface desiccation and to reduce friction. All are viscoelastic with non-newtonian flow properties, i.e. reduction of viscosity when shear rate increases and inversely, as this is also the case of normal human tears (Cowman et al., 2015; Petricek et al., 2008; Pouliquen, 1999). These treatments are effective to improve tear film stability, and corneal and conjunctival staining (Springs, 2010; Johnson et al., 2008).

6.2. Lubricin

Lubricin (Proteoglycan 4) is a large glycoprotein with a central mucin-like domain. It is synthetized and secreted by chondrocytes and synoviocytes and play an important role in protecting articular cartilage. Lubricin has recently been discovered at the ocular surface, where it functions as a boundary lubricant and appears to play a protective role by reducing friction between the human cornea and conjunctiva (Schmidt et al., 2013). Recombinant human lubricin was recently found to provide significantly greater symptom and ocular sign reduction compared to sodium hyaluronate 0.18% eye drops in patients with moderate DED (Lambiase et al., 2017).

6.3. Mucin secretagogues

6.3.1. Diquafosol tetrasodium

Diquafosol tetrasodium (DQS) is a purinergic P2Y2 receptor agonist, which facilitates mucin production and tear secretion, and is approved as an ophthalmic solution at 3% concentration in some Asian countries (e.g. Japan and south Korea), but not yet approved in the US and Europe. DQS exerts its effects on the conjunctival epithelium and GCs and promotes the secretion of both water and mucin, thereby stabilizing the tear film (Garcia-Zalisnak et al., 2014). DQS upregulates the expression of secreted (MUC5AC) and membrane-bound mucins (MUC1, MUC4, and MUC16). In several studies, topical application of DQS 3% improved signs and symptoms of dry eye, including SS-ADDE, NSS-ADDE and post-operative dry eye (Jeon and Hyon, 2016; Baek et al., 2016; Mori et al., 2014). Increased mucin production by addition of DQS potentially improved the tear film stability and reduced ocular

symptoms in patients who had persistent dry eye after LASIK (Mori et al., 2014).

6.3.2. Rebamipide

Rebamipide is a quinolone derivative used as a cytoprotectant for the treatment of gastric mucosal disorders and gastritis (Fujioka et al., 2003). It has recently been developed as an ophthalmic solution for the treatment of DED and was shown to improve dry eye symptoms and ocular surface signs including corneal and conjunctival staining and TBUT after 4 weeks of treatment in a randomized, placebo-controlled study (Kinoshita et al., 2012, 2013). Previous studies in rabbits have established that topical application of rebamipide increase both the number of GCs and the level of mucin in the bulbar conjunctiva (Urashima et al., 2004, 2012). Rebamipide has anti-inflammatory and antioxidant properties (Ohguchi et al., 2013) and was shown to induce the proliferation of cultured rat conjunctival GCs (Rios et al., 2006) and to increase the number of GCs in the lid wiper in *ex vivo* experiments in human (Kase et al., 2017). In vitro, MUC16 expression was also increased in human corneal epithelial cells but not conjunctival epithelial cells with rebamipide treatment (Uchino et al., 2016). Topical rebamipide was shown to enhance conjunctival GC recovery after vitrectomy (Kato et al., 2016) and to block the reduction of GCs when used during cataract surgery (Kato et al., 2017). Topical rebamipide also improved SLK in patients with thyroid eye disease, and was suggested as a first-line treatment in such patients (Takahashi et al., 2014).

6.3.3. Vitamin A

GC loss has long been identified as a hallmark of systemic vitamin A deficiency (Sommer, 1983). Previous animal studies have demonstrated an improvement in conjunctival histology with the reappearance of GCs, a reduction in surface keratinization and even a reversal in squamous metaplasia with the use of topical retinoic acid (Tseng, 1986). In a dry eye rabbit model whose lacrimal glands were resected, topical application of retinyl palmitate (1500 IU/mL) showed greater improvement than 0.1% hyaluronic acid in both fluorescein and rose bengal scores and in the density of conjunctival GCs (Odaka et al., 2012).

In a rat dry eye model, retinol palmitate was shown to promote corneal MUC4, and conjunctival MUC5AC and MUC16 gene expression compared to vehicle treated eyes (Tabuchi et al., 2017). In a randomized controlled clinical trial, topical vitamin A produced significant increase in GC density compared to artificial tears (Kim et al., 2009). It was suggested that vitamin A improves mucin abnormality in corneal and conjunctival cells by promoting mucin expression and recovery of GCs (Toshida et al., 2017).

6.4. Immunomodulators

6.4.1. Cyclosporine A

Topical Cyclosporine A (CsA) is currently designated for the treatment of inflammation in DED. It was shown to inhibit conjunctival epithelial apoptosis and protect against GC loss in experimental murine DED model (Strong et al., 2005) and increase GC density in dry eye patients (Kunert et al., 2002; Pflugfelder et al., 2008). In a 3-month randomized controlled study, CsA led to a significant improvement of ocular symptoms and signs including GC density and impression cytology grade compared to carboxymethyl cellulose eye drops (Kim et al., 2009). In another clinical trial, topical CsA significantly increased GC density after 4 months of treatment in patients with dysfunctional tear syndrome compared to preservative-free hydroxyl methyl cellulose eye drops (Demyray et al., 2011). In patients with NSS-ADDE or SS-ADDE, topical CsA for 6 months was shown to be effective in increasing GC density and tear film stability in patients with grade 1 DED (i.e. dry eye symptoms without corneal staining) or grade 2 DED (i.e. ocular symptoms and corneal staining, punctate keratitis and filament), but not in patients with grade 3 DED (i.e. corneal scar and neovascularization) (Yuksel et al., 2010). At the same time, a 6-month treatment significantly decreased HLA-DR expression as a marker of surface inflammation in patients with moderate to severe DED (Brignole-Baudouin et al., 2001).

6.4.2. Lifitegrast

Lifitegrast (SAR 1118) is an inhibitor of adhesion, migration, activation, and recruitment of T cells by blocking LFA-1/ICAM-1 interaction (Zhong et al., 2012; Abidi et al., 2016). It was shown to inhibit the release of cytokines including IFN-γ, IL-2, IL-4, and IL-17 from activated blood lymphocytes. Efficacy and safety was demonstrated in a dose-response tolerability study in dogs suffering with DED (Murphy et al., 2011). Although the basic effects on GCs have not yet been investigated, similar effects based on the similarity of action with CsA could be expected.

6.4.3. Nerve growth factor and Tavilermide

Topical application of NGF eye drops was proposed as a new treatment approach in DED since NGF is potentially capable of restoring ocular surface homeostasis by improving corneal sensitivity and reflex tear secretion (Lambiase et al., 2011). Besides various functions including modulation of inflammatory reactions by inhibition of cytokine and chemokine release and leukocyte extravasation, in promoting regeneration of sensory and autonomic nerve fibers and in regulating tear film production by lacrimal gland, NGF was shown to modulate conjunctival epithelial cell differentiation into GCs and to promote ocular surface healing by stimulating corneal and conjunctival epithelial cell proliferation (Mantelli et al., 2013b). Tavilermide (MIM-D3) is a tyrosine kinase TrkA receptor agonist that functions as a peptidomimetic of NGF. In vitro studies in cultured primary rat conjunctival cells demonstrated that Tavilermide stimulated mucin secretion (Jain et al., 2011). Early clinical evaluation of Tavilermide in individuals with dry eye indicated significant improvements in signs and symptoms compared with placebo (Meerovitch et al., 2013).

6.4.4. Mycophenolate mofetil

Mycophenolate mofetil (MMF) is an inhibitor to T and B lymphocytes, which has been reported to be effective in the treatment of transplant rejection and multiple autoimmune diseases. MMF was shown to induce proliferation and MUC5AC mRNA expression in human conjunctival GCs *in vitro* (He et al., 2010). Further research is needed to confirm the potential effect of topical MMF to control inflammation and enhance GC function in patients with DED.

6.5. Mucolytics

Dehydration and subsequent mucus accumulation may be the counterpart to the positive effects of mucins (Fraunfelder et al., 1977). Topical 5% N-acetylcysteine (NAC), given 2–4 times daily, can be used to treat DED, filamentary keratitis and corneal mucus plaques because of its mucolytic properties, specifically reducing disulphide bonds, which are involved in mucus formation (Thode and Latkany, 2015). NAC is an acetylated derivative of the natural amino acid l-cysteine. It has mucolytic, anti-inflammatory and antioxidant properties. NAC regulates mucus secretion and reduces mucus accumulation in the conjunctival sac in patients with dry eye. It was reported to reduce subjective symptoms in DED patients (Pokupec et al., 2005) and to improve sign and symptoms in patients with MGD (Akyol et al., 2010, 2012). NAC was also shown to improve TBUT and mucous fern pattern in patients with blepharitis (Yalcin et al., 2002).

Oral mucolytics (i.e. ambroxol, bromhexine) were also shown to improve dry eye symptoms in SS-ADDE patients (Ichikawa et al., 1988). But conversely, oral ambroxol can disturb tear film and ocular surfaces by attenuating the mucin layer of the tear film in patients with no ocular surface disease (Kim et al., 2013).

7. Future directions and conclusions

There is a large variety of ocular mucins with a common function to protect the corneal and conjunctival epithelium from pathogens and other environmental and cellular contaminants. Some mucins (MUC5AC, MUC1, MUC4, MUC16) are rather well characterized but others need to be investigated in more detail in order to enhance our understanding of the ocular mucin homeostasis. There is also some evidence that mucins play a role in cell signaling transduction and intercellular communication with a particular cross-talk with the immune system. Although the mechanisms are not well known, mucin expression and secretion including soluble membrane-shed mucin seems to be tightly controlled. Little is known about the live cycle of GCs but considerable advances have been made with the identification of the transcriptional factor Spdef in GC differentiation (Gipson, 2016). Even less is known about the role of conjunctival GCs that do not contain mucin products, while there is some evidence of their predictive role in bleb filtration outcome (Matropasqua et al., 2017). Even though in this case aqueous humor is the likely explanation for "empty" GCs, many other conditions support the hypothesis that GCs may secrete or contain other components, like TGF, and therefore play a central role in immunoregulation of the ocular surface. As GCs are highly sensitive to chronic inflammation, an autonomous vicious cycle can be proposed, namely higher inflammation, reduction in GCs leading to lesser GC secretion resulting in higher, or alternatively, longer inflammation.

Thus, mucin dysfunction can be considered as a hallmark of DED and is present in other ocular surface inflammatory disorders (Fig. 11). There is compelling evidence that mucin dysfunction is closely related to ocular surface immune and inflammatory reactions.

Nevertheless, it is difficult to differentiate between abnormalities in ocular surface disease (DED or others) and their cause. According to the ocular surface disease, the cause may be different but lead to the same self-stimulated cycle. Some diseases may lead to earlier and more severe inflammatory changes (as in Sjogren syndrome) while in other cases, inflammation may appear most likely as a secondary mechanisms following the accumulation or repetition of risk factors and aggressions (e.g., menopause, environment, contact lens or preservative use).

Two different situations are to be distinguished 1) a proliferation of GCs and increased release of mucin secretion by proliferating GCs in acute phase of inflammation and 2) a loss of GCs and mucin secretions with advanced/persistent chronic inflammation. Mucins alteration may be the first sign of ocular surface disease and thus should be adequately investigated. Changes in mucins can lead to ocular discomfort even in mild DED and loss of GC mucin secretion can promote epithelial stress, squamous hyperplasia, epithelium stratification and keratinization as shown in severe DED.

In conclusion, mucin deficiency is a common, but poorly recognized cause of DED and at present, is not routinely evaluated in a clinical practice setting. Being able to precisely assess the level of mucin impairment would be extremely useful for the ophthalmologist, since topical treatments dedicated to mucin layer restoration are now becoming available. Therefore research on mucin secretion and/or mucin-secreting cells may bring valuable information and new perspectives for both research on diagnostic biomarkers and the management of an array of ocular surface disorders.

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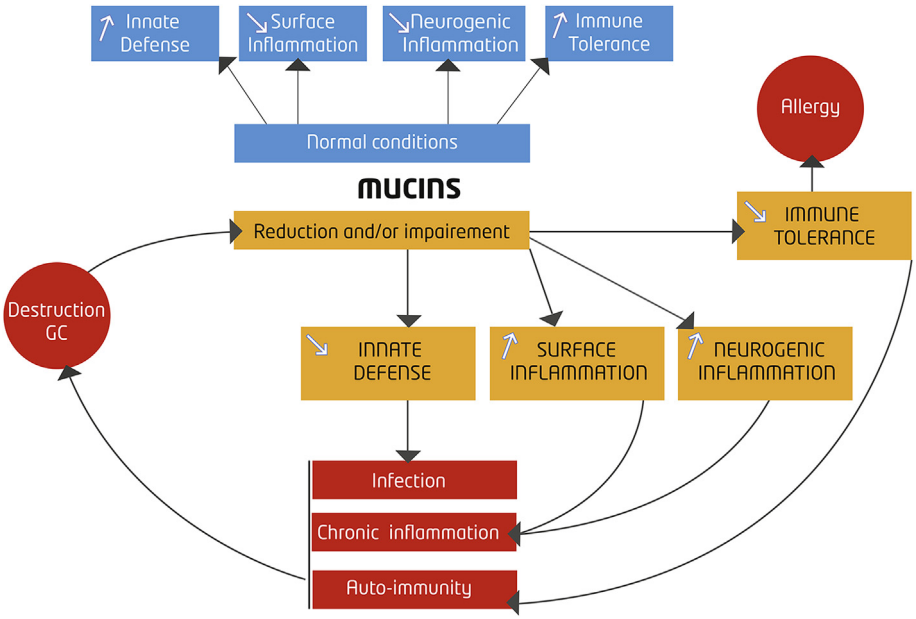

Fig. 11. Proposal role for mucin and goblet cells in ocular surface homeostasis.

BioMedical laboratory in Quinze-Vingts for providing the cytological pictures of goblet cells and cell cultures.

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