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REVIEW

The tear film and ocular mucins

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Abstract

The trilaminar tear film, composed of the lipid, aqueous and mucin layers, has many functions including defending the ocular surface. The aqueous layer has several soluble antimicrobial factors that protect the ocular surface. Ocular mucins have recently been studied with regard to their role in the defense of the eye as well as in dry eye syndromes. To date, 15 mucin genes have been identified, and six of these mucin genes are localized to or secreted by ocular glands or epithelia. Understanding the production, secretion and function of ocular mucins will aid in the treatment of dry eye syndromes and ocular surface microbial infections.

Key Words: mucin, tear film

INTRODUCTION

The tear film has a unique three-layer composition that enables it to perform many functions. The seven major functions of the tear film are:[1] maintaining a smooth surface for light refraction;[2] lubricating the eyelids;[3] lubricating the conjunctiva and the cornea;[4] supplying the cornea with nutrients and transporting metabolic by-products from the corneal surface;[5] providing white blood cells with access to the cornea and conjunctiva;[6] removing foreign materials from the cornea and conjunctiva; and[7] defending the ocular surface from pathogens via specific and nonspecific antibacterial substances.[1]

The purpose of this paper is to review the components and functions of the tear film, with an emphasis on the mucus layer and ocular mucins. The components of the tear film, especially the antimicrobial properties of the aqueous layer, have been studied. More recently, the ocular mucins have been the subject of considerable study with a focus on their previously unrecognized antimicrobial properties and role in dry eye syndromes. In the future, mucins may be key in the treatment or prevention of ocular surface microbial infections and dry eye syndromes.

The tear film

The eye has several defenses in addition to the tear film including the bony orbit, eyelids, cilia, corneal epithelium and conjunctival epithelium.[2] The ocular surface is in constant contact with microorganisms but rarely becomes colonized or infected with these agents because of these ocular defenses, especially the tear film. Tears are composed of three layers: the outermost lipid layer, the middle aqueous layer and the innermost mucus layer.[3]

Recently, the thickness of the human tear film, previously thought to be only 7 μm, has been re-examined.[4,5] Recent studies suggest that the tear film is actually 35–45 μm thick with the mucus layer comprising 30 μm[4,5] Verification of the actual tear film thickness will require additional studies.[6,7]

The meibomian layer

The meibomian oil layer provides a smooth optical surface, reduces the evaporation of tears, and prevents contamination of the tear film from debris.[8] This oil layer is produced by the meibomian glands, which are modified sebaceous glands located in the tarsal plates.[8] Meibum has a lower melting point than sebum, which allows it to remain a fluid when in the tear film.[8] Meibum consists of both polar and nonpolar lipids, and is composed mostly of nonpolar sterol esters and wax.[8–10] Acting like a surfactant, the polar fraction of the meibomian layer, comprised mostly of phospholipids, spreads over the aqueous layer of the tear film while the nonpolar fraction of the meibomian layer lies more superficial.[8,11]

The surface tension of the tear film decreases when lipids spread over the surface. The decrease in surface tension draws water into the tear film and thus increases the film thickness. This decrease in surface tension also allows the lipids to continue to spread during blinking.[8] The removal of the meibomian oil layer leads to evaporation of the tear film, resulting in decreased tear film break-up time and increased

tear osmolarity.[8,12] Increased tear osmolarity is believed to be relevant in the pathogenesis of various dry eye conditions.[12]

The meibomian glands are sebaceous glands that undergo holocrine secretion, which means that the entire cell and its contents are released. Therefore, regulation of meibum secretion occurs through modulation either of lipid synthesis or of cell maturation. Androgens are known to regulate other nonocular sebaceous glands. Androgen receptor mRNA and protein have been localized to rat, rabbit and human meibomian acinar epithelial cells.[14,15] In those studies, it was determined that androgens did modify the lipid production of the meibomian gland.[14,15] It is hypothesized that androgens stimulate meibomian secretion whereas estrogens reduce secretion.[10,14]

The meibomian glands have autonomic innervation, and contain various neuropeptides; however, no direct evidence exists of either sympathetic or parasympathetic control of secretion.[8,16] The neuropeptides calcitonin gene-related peptide, substance P, neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) have been identified by immunocytochemistry in human and guinea pig meibomian glands in association with blood vessels.[16] Additionally, nerve fibers have been identified surrounding the meibomian alveoli. The actions of these neuropeptides and nerves on the meibomian gland have not been identified.[16,17]

The aqueous layer

The aqueous portion of the tear film is active in the lubrication and protection of the ocular surface. It washes away foreign materials introduced to the conjunctiva or cornea and contains antibacterial factors including immunoglobulins. The aqueous layer also contains soluble mucin that decreases the surface tension, enhances the spread and coherence of the aqueous layer, and contributes to the viscosity of the tear film.[18]

The aqueous layer also contains lactoferrin, lysozyme, secretory immunoglobulin A (sIgA), immunoglobulin G (IgG), immunoglobulin M (IgM), albumin, transferrin, ceruloplasmin, tear specific prealbumin, and glycoproteins, which participate in the defense of the ocular surface.[19,20]

Nutrients provided to the avascular cornea by the aqueous layer include inorganic salts, glucose, oxygen, and proteins.[19] The aqueous portion of the tear film is 98.2% water and 1.8% solids with proteins constituting most of the solids.[19] Tears contain detectable levels of amino acids, bicarbonate, calcium, urea and magnesium.[19]

In most animals, the lacrimal gland and the gland of the third eyelid produce the aqueous layer. These are tubuloacinar glands with ductules that deliver tear secretions to the conjunctival fornices. The Harderian gland contributes to the aqueous tear production in some species including cattle, rodents and birds.

The lacrimal gland is affected by the parasympathetic and sympathetic nervous systems as well as various hormones.[21–23] Stimuli from the cornea, conjunctiva, optic nerve and brain stimulate lacrimal gland fluid secretion using parasympathetic and sympathetic efferent pathways.[13] The acinar cells, duct cells and blood vessels of the lacrimal gland are innervated by parasympathetic and sympathetic nerves.[13] Parasympathetic nerves contain acetylcholine and vasoactive intestinal peptide (VIP), while sympathetic nerves contain norepinephrine and neuropeptide Y (NPY). Sensory nerves from terminal branches of the trigeminal nerve contain substance P and calcitonin gene-related peptide. The parasympathetic neuropeptides increase tear secretion through a G protein pathway and perhaps a Ca^{2+}/calmodulin pathway.[13,24] Sympathetic stimulation increases tear secretion by affecting the vascular supply to the lacrimal gland as well as activating a G protein pathway.[21,24] Lacrimal gland secretion is inhibited by Leu-Enkephaline (L-Enk), a neuropeptide that interacts with inhibitory G proteins, thus interfering with the activation of adenylate cyclase by the G stimulatory proteins.[13]

Androgens and estrogens modulate lacrimal gland secretion. A lack of androgen causes reversible degenerative changes in lacrimal tissue, a decreased total volume of tears, and decreased protein content of tears. In humans with keratoconjunctivitis sicca (KCS) or Sjögren's syndrome, systemic androgen increases tear volume. The effect of estrogen on the lacrimal gland is controversial. Estrogen deficiency has been linked to the development of KCS as well as degeneration of the lacrimal gland. Other studies have shown no change in the lacrimal gland or tear film with estrogen deficiency.[22,25]

The mucus layer

Ocular mucus is composed of mucin, immunoglobulins, urea, salts, glucose, leukocytes, cellular debris and enzymes.[26] The mucus layer lubricates and protects the cornea, anchors the aqueous tear film to the corneal epithelium protecting it from shear forces, and prevents dessication and bacterial contamination.[27] The corneal epithelium is hydrophobic, so the hydrophilic layer, created by the mucus, facilitates the spread of the aqueous layer evenly over the ocular surface. The mucus layer is not tightly adhered to the epithelial layer, but rather attaches to the glycocalyx and undergoes free movement across the cornea.[28] This allows the mucus to spread evenly across the cells and prevents damage to the epithelium during blinking.[29]

Mucus may prevent the attachment of bacteria to the ocular surface as well as concentrate IgA at the mucosal surface.[30] Mucus entraps material, engulfs it in a mucus thread and moves the entire thread to the lower fornix where it is expelled from the eye.[2,31] This mechanism allows the eye to constantly remove cellular debris, micro-organisms and foreign material.[31] The continual replacement and removal of tears also help to inhibit bacterial adherence to the corneal and conjunctival epithelia.

A deficiency of aqueous tears, damage to the epithelium or glycocalyx, or an increase in epithelial cell loss, allows mucus to adhere to itself or to the epithelium causing mucus clumping and leads to tear film instability and corneal damage.[28,31] Ocular mucus lubricates and hydrates the epithelial cells, lowers the surface tension and increases stability of the tear film.[28] The mucus layer and ocular mucins will be discussed further in a later section.

The mucus layer is secreted mostly by the conjunctival goblet cells; however, the corneal and conjunctival epithelium also contribute to the mucus layer. Conjunctival goblet cells are secretory apocrine cells. Once the goblet cell is stimulated, subsurface vesicles containing glycoproteins fuse with the limiting membrane of the goblet cell.[29] As the goblet cell emits mucin and glycoproteins onto the cornea, the cell loses its basement membrane connections and desquamates. Additional sources of ocular mucin will be discussed in a later section.

Goblet cells may be stimulated to secrete mucin by histamine, antigen, immune complexes or mechanical action (i.e. blinking).[30] Direct and indirect neural control of mucin secretion has also been described.[32] Sensory, sympathetic and parasympathetic nerves innervate the conjunctiva surrounding goblet cells.[33] Thus, stimuli from the cornea and conjunctiva can indirectly induce goblet cell mucus secretion through diffusion of neuropeptides from these nearby nerves.[13,32–34] Muscarinic and α-adrenergic receptors are present on immature goblet cells and may directly regulate secretion.[34]

The glycocalyx

The corneal and conjunctival epithelium is covered with microvilli and microplicae, which in turn are covered by a glycocalyx that is composed of glycoproteins and glycolipids.[29,35,36] This glycocalyx extends from the microvilli and microplicae approximately 300 nm, can be angular and branching, and can extend laterally between microvilli.[35] Each filament branches distally and is associated with the cell membrane. The mucus layer of the tear film attaches to the carbohydrate-rich glycocalyx.[37] This attachment of mucus may protect the epithelium by causing the shear forces of blinking to break up the mucus layer further away from the cell surface. The attachment of mucus to the glycocalyx also allows the aqueous layer to spread evenly over the corneal epithelium.[29]

ANTIMICROBIAL SUBSTANCES IN TEARS

Tears contain both nonspecific and specific antimicrobial substances, most of which are found in the aqueous layer. Lysozyme, lactoferrin, α-lysin and complement proteins are nonspecific antimicrobial substances.[38]

Lysozyme is found in most mammal, avian and insect tears; however, lysozyme activity has not been detected in cattle.[39] Lysozyme is secreted by the lacrimal gland and is considered a first line of defense against ocular pathogens.[38] Lysozyme causes bacteriolysis through the hydrolysis of peptidoglycan which forms the bacterial cell wall.[40] Due to its chitinase ability, lysozyme also has antifungal properties.[38]

α-lysin causes cell membranes to rupture by an unknown mechanism of action. The concentration of α-lysin in tears is greater than its concentration in serum, plasma or aqueous humor.[41] The source of α-lysin in the tear film has not been determined.[42]

Lactoferrin has a high concentration in tears (65–160 mg/mL), is secreted by the lacrimal glands, and has been identified in human, cattle and other mammal tears.[38,43,44] Lactoferrin reversibly binds two atoms of iron, thus depleting the iron essential for bacterial metabolism and growth.[43] Some bacteria can overcome the effect of lactoferrin by expressing outer membrane proteins or lactoferrin receptors that bind lactoferrin, thereby allowing the bacteria to utilize iron bound to lactoferrin.[45] This ability to chelate iron from the host environment may increase the virulence of some bacteria. Lactoferrin also binds copper, IgA, IgG and complement proteins, thereby modulating the immune system.[42]

IMMUNOGLOBULINS IN TEARS

Specific antimicrobial substances in tears include secretory IgA, IgG and IgM.[20] IgA is considered the primary immunoglobulin of the tear film.[38] Normal human tears contain 10–80 mg of IgA per deciliter.[46] Secretory immunoglobulin A is secreted by the plasma cells located in the interstitium of the lacrimal glands as well as by the substantia propria of the conjunctiva.[38] Secretory IgA is different from serum-derived IgA because it is composed of two IgA molecules attached by a J chain. This protects sIgA from the proteolytic enzymes in tears.[42] The secretory component is produced by the acinar epithelial cells while the plasma cells produce the J chain. The secretion of IgA by lacrimal tissue is probably regulated by hormones, immune factors and neural responses.[47]

Secretory IgA protects the eye from viral infection, bacterial attachment and colonization, and parasite infestation.[47] Secretory IgA is believed to coat micro-organisms, thereby preventing bacterial adherence to the corneal epithelium by causing bacterial agglutination, antimicrobial neutralization and lysis.[30,38] IgA is found free in the tears, as well as bound to ocular mucus or free glycoproteins.[35] Immunoglobulin G is present in very low concentrations in the tears.[38,46] Its concentration increases during inflammation and it participates in phagocytosis and complement-mediated bacterial lysis.[38] Immunoglobulin M is also present in very low concentrations in tears.[20,48]

OCULAR MUCIN

In the past most of the research regarding the antimicrobial factors of the tear film have focused on the aqueous layer and its components. Investigators are now beginning to investigate the mucus layer and its antimicrobial properties. The mucus layer and ocular mucins stabilize the tear film, provide a smooth refractive surface over the cornea, and lubricate and prevent dessication or bacterial contamination of the cornea and conjunctiva.

Mucins are glycoproteins expressed by epithelial tissues of mucosal surfaces. They protect tissues by functioning as antioxidants, providing lubrication, and inhibiting bacterial adherence. To date, 15 mucin (MUC) genes have been identified (see Table 1),[32,49] six of which are associated with the tear film. Mucins are classified as either secretory or membrane bound, and the tear film and ocular epithelium contain both types.[50]

Table 1. Mucin genes and their association with the eye

MUC gene	Type	Association
MUC1	Membrane bound	Tear film, conjunctival epithelium, corneal epithelium, lacrimal gland
MUC2	Secretory, gel forming	Tear film, conjunctival epithelium/goblet cell
MUC4	Membrane-bound, soluble form	Tear film, conjunctival epithelium, corneal epithelium, lacrimal gland
MUC5AC	Secretory, gel forming	Tear film, conjunctival epithelium/goblet cell
MUC5B	Secretory, gel forming	Conjunctival goblet cell, lacrimal gland
MUC7	Secretory, soluble	Tear film, conjunctival epithelium, lacrimal gland

Characteristics of mucin

Ocular mucus is composed of mucin, immunoglobulins, urea, salts, glucose, leukocytes, cellular debris and enzymes.[26] Mucins have a molecular weight ranging from 5×10^4 to over 4×10^6 kDa.[50,51] They consist of a protein core, oligosaccharide side chains, and nonglycosylated peptides linked to a glycosylated portion by disulfide bonds.[51,52]

MUC1, MUC2, MUC4, MUC5AC and MUC7 are associated with the tear film (see Table 1), and transcripts of those mucins are found in normal human conjunctival epithelium.[27,53–57] MUC1 and MUC4 are produced by the conjunctival epithelium;[27,58] MUC1 is also expressed by corneal epithelial cells.[54,56,57,59]

MUC5AC, MUC5B and MUC2 are associated with goblet cells.[27,58,60,61] Conjunctival goblet cells are the source of MUC5AC, a soluble ocular mucin.[27,58] MUC1, MUC4, MUC5B and MUC7 are associated with the lacrimal gland.[62] Recently, it was shown that MUC4 is synthesized by the lacrimal gland.[62]

Origin and secretion of mucin

MUC5AC, MUC5B and MUC2 are secretory and MUC1 and MUC4 are membrane bound or membrane spanning.[50,59] Secretory mucins form large oligomers through linkage of protein monomers via disulfide bonds at their N- and C-termini.[53] Secretory mucins are further categorized as gel-forming or soluble.[63] The former type form a gel overlying the epithelial surface that provides lubrication and protection to the cells. However, there are soluble secretory mucins, like MUC7, that have smaller peptide backbones, and do not form a gel.[50,53,54,64]

Membrane-bound mucins have a hydrophobic amino acid segment that spans the plasma membrane, allowing the mucin core protein to remain intimately associated with the epithelial cell.[64] The membrane-bound mucin extends 200–500 nm above the cell surface, thus interfering with receptors and antigens located on the glycocalyx.[57,64] Membrane-bound mucin does not form an extracellular gel and disulfide links between peptide monomers have not been described.[51] MUC4 is generally considered a membrane-bound mucin; however, a soluble or secretory form of MUC4 (without the -COOH terminal domain) has been identified in the lacrimal gland, tears, milk, and in the colon.[62,65]

Currently, four sources of ocular mucin have been proposed including the conjunctival goblet cells, glycocalyx, conjunctival and corneal epithelial cells, and the lacrimal gland. The majority of the ocular mucin in the tear film is secreted by vesicles found in the secretory apocrine conjunctival goblet cells. Soluble mucins in the tear film may also originate from membrane-bound mucin released from the glycocalyx.[32] Conjunctival and corneal epithelial cells also secrete membrane-bound mucin.[32,50,63] The lacrimal gland is being investigated as a source of soluble and membrane-bound mucin.[32,50,62]

Regulation of secretion of ocular mucin is an area of current research. The ocular surface epithelium has a requirement for vitamin A.[66] A deficiency of vitamin A causes loss of the corneal microvilli and reduces the number of conjunctival goblet cells.[61,66] Rats fed a diet deficient in vitamin A for 20 weeks develop keratinization of the entire conjunctival epithelium and a complete absence of goblet cells.[61] This study suggests vitamin A indirectly regulates mucin production.

The rat gene ascites sialoglycoprotein (ASGP), also known as sialomucin complex (SMC), is a homolog to the human mucin gene MUC4.[61] In a study by Tei *et al.*, after 20 weeks of vitamin A deprivation, ASGP was no longer detected in the rat cornea or conjunctiva and rMUC5AC was no longer detected in the conjunctival epithelium. These data suggest that ASGP (MUC4) and rMUC5AC are regulated by vitamin A.[61] The characterization of the regulatory region of mucin genes is needed to assess the direct or indirect regulation of mucin by vitamin A.

In a study by Jumblatt,[67] rabbit and human conjunctiva were assayed for the effects of purine and pyrimidine nucleotides on mucin secretion and therefore evidence of $P2Y_2$ nucleotide receptors. Adenosine 5′triphosphate (ATP) and uridine triphosphate (UTP) stimulated mucin secretion when incubated with nictitans tissue of a rabbit. The study concluded that rabbit and human conjunctival tissues do contain $P2Y_2$ receptors that are activated by ATP and UTP to secrete mucin. $P2Y_2$ receptors belong to the G protein-coupled metabotropic receptor superfamily.[67] In the future this pathway may be pharmacologically regulated to treat dry eye syndromes.

An increase in total mucin secretion and an increase in MUC5AC secretion by human conjunctival tissue has been noted when it was incubated with ionomycin. This suggests that MUC5AC secretion is stimulated by an intracellular influx of calcium, or that this ion may serve as a common intracellular pathway for mucin secretion in response to various stimuli.[68]

BACTERIAL ADHERENCE AND MUCIN/MUCUS

Ocular mucus protects against bacterial adherence to the corneal epithelium, and alterations in mucus production, composition or clearance promote bacterial adherence to the cornea. In a study by Fleiszig *et al.*,[69] removal of corneal mucus increased the adherence of *Pseudomonas aeruginosa* to rabbit corneas by 3–10-fold. When porcine stomach mucin, bovine submaxillary gland mucin, or ocular mucus was added to normal corneas, it significantly reduced bacterial adherence with levels as low as 35 μg/mL. However, only ocular mucus or mucin purified from ocular mucus decreased bacterial adherence to the injured corneas, and the nonmucin fraction did not affect bacterial adherence on either intact or injured corneas.[69]

Mucins may protect epithelial surfaces directly (i.e. through specific receptors) by binding pathogens before they attach to the corneal epithelium, or indirectly by inhibiting microbial colonization by competitively blocking microbial receptors found on the epithelium.[70] Some mucins, like MUC1, extend above the cell membrane preventing the approach of micro-organisms to cell attachment sites.[71] Mucins may nonspecifically bind pathogens by merely entrapping pathogens. The MUC1 mucin product is highly negatively charged because of its sialic acid residues.[64] This negative charge may directly repel pathogens from the epithelial surface[72] and lead to repulsion between molecules, thus enhancing the movement of mucus across the corneal epithelium.[64]

Owing to a high sialic acid content, ocular mucins may bind to and provide antiviral effects. Binding of influenza, rota-, and coronavirus have previously been established for intestinal mucin.[32] It is also believed that sialic acid competitively blocks the attachment of bacterial pathogens by binding to bacterial adhesins located on the epithelial cell and thus preventing bacteria from binding to the cells. They may bind to corneal epithelial bacterial receptors, thus competitively blocking the adherence of bacteria.[32,71,72] Epithelial corneal binding sites have been identified for *Pseudomonas aeruginosa*, so it is possible for mucin to protect against *Pseudomonas* sp. infection by competitive inhibition.[32,72]

MUCIN AND DRY EYE SYNDROMES

Keratoconjunctivitis sicca (KCS) is a common ocular disease in dogs resulting from a deficiency in the aqueous tear film characterized by a mucoid to mucopurulent conjunctivitis, pain, keratitis, corneal ulcers and blindness.[73,74] It has been suggested that KCS may predispose the ocular surface to infection. The normal flora of the canine conjunctiva consists of gram-positive organisms, mainly *Staphylococcus* species, and some gram-negative organisms such as *Neisseria* species.[75,76] The normal flora of dogs suffering from KCS includes many pathogenic organisms such as coagulase positive *Staphylococcus* species and beta hemolytic *Streptococci*, as well as *Pseudomonas* species. These dogs also have heavier growth of organisms compared to normal dogs.[75] Dogs that respond to topical cyclosporine treatment, indicated by an increase in Schirmer tear test values, have a significant decrease in corneal bacterial isolation.[77]

In addition to quantitative tear film deficiency, qualitative deficiencies are also associated with KCS. Many mucin properties are abnormal in KCS including mucus accumulation,[78] alteration of mucus production pathways,[79] decreased conjunctival goblet cell density,[80–83] and altered mucin expression and glycosylation.[84]

Hicks *et al.* report differences in glycosylation and electrophoretic mobility between ocular mucus samples from dogs with KCS and normal dogs.[84] These changes were attributed to alterations in tear film osmolarity, pH and composition, as well as possible alterations in the regulation of goblet cell differentiation, mucin gene expression, and synthesis and glycosylation of mucins.

A recent molecular study by Argueso *et al.* reported a decrease in the level of MUC5AC protein in the tear fluid of patients with Sjögren's syndrome compared to normal individuals.[49] A deficiency in the expression of MUC5AC followed by a decrease in the amount of mucin in the tear film may lead to tear film instability and resultant pathologic changes in the ocular surface.

A study by Moore *et al.* describes the effect of Cyclosporine A (CsA) on the conjuctival mucin levels of dogs with bilateral keratoconjunctivitis sicca (KCS), experimentally induced by surgical lacrimal gland removal.[85] After 2 weeks of twice-daily topical 2% CsA treatment, the mucin levels returned to control levels, and the degree of conjunctivitis and ocular discharge were decreased, even though the Schirmer tear test values remained below normal. This finding suggests that in dogs with KCS, CsA increases mucin production. Further investigation of the role of mucins in canine dry eye conditions is warranted.

CONCLUSION

The tear film provides a smooth surface for light refraction, nourishes the cornea and plays an important role in the ocular defense system. Deficiency in the amount of tear production or alteration in tear composition can lead to ocular pathology. A deficiency in mucin production can lead to decreased tear break-up time and ocular disease. Using specific and nonspecific methods, the epithelial cell, glycocalyx and the tear film work together to protect the eye from disease. Increased understanding of the production, secretion and function of ocular mucins will enhance treatment of dry eye syndromes and ocular microbial infections.

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