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Dry Eye Disease and Microbial Keratitis: Is There a Connection?

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Abstract

Dry eye is a common ocular surface disease of multifactorial etiology characterized by elevated tear osmolality and inflammation leading to a disrupted ocular surface. The latter is a risk factor for ocular surface infection, yet overt infection is not commonly seen clinically in the typical dry eye patient. This suggests that important innate mechanisms operate to protect the dry eye from invading pathogens. This article reviews the current literature on epidemiology of ocular surface infection in dry eye patients and laboratory-based studies on innate immune mechanisms operating at the ocular surface and their alterations in human dry eye and animal models. The review highlights current understanding of innate immunity in dry eye and identifies gaps in our knowledge to help direct future studies to further unravel the complexities of dry eye disease and its sequelae.

Keywords

antimicrobial peptides; contact lens; cornea; conjunctiva; cyclosporine; dry eye; infection; innate immunity; ocular surface inflammation; tears

I. Introduction

Dry eye is defined as a multifactorial disease that is caused by a decrease in tear production or an increase in tear evaporation and is associated with elevated tear osmolarity and symptoms of ocular irritation.[1] Patients often report a variety of symptoms, including ocular burning, itching, foreign body sensation, photophobia, redness, and reduced visual acuity.[2] Dry eye is thought to be one of the most common causes for a patient to consult an eye care professional.[3] Prevalence rates are greatest among women and the elderly population,[4] with various epidemiological studies estimating a 5–30% prevalence of dry eye in people who are 50 years and older.[2] Dry eye often has a significant impact on the patient's visual function and overall quality of life,[5–7] and the use of various therapeutic and palliative treatment options imposes a major economic burden on the patient.[4, 8, 9] Treatment can be

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challenging, as patients may present with ocular surface pathology but have no or few symptoms.[10] Numerous factors have been associated with dry eye disease, such as poor systemic health, concurrent ocular disease, use of drying systemic medications, and the external environment.[2]

In dry eye, a chronic inflammatory reaction, possibly subclinical, is generated at the ocular surface, which can result in vital dye staining of the cornea and conjunctiva.[11] The accumulation of inflammatory molecules at the ocular surface of dry eye patients,[12–15] accompanied by a stagnant tear film[15] and decreased level of mucins,[16] can lead to destruction of epithelial tight junctions, and result in sloughing of the ocular surface epithelia.[17] Epithelial anatomy (tight junctions, in particular) is one of several so-called innate immune response mechanisms that play a crucial role in preventing invasion of microorganisms into the ocular surface.[18–20] Innate responses are the first line of defense against infection, responding immediately but in a nonspecific manner to invading pathogens.[21, 22] Mucous membranes (eg, conjunctiva), secreted proteins (eg, lysozyme), mechanical barriers (eg, closure of eyelids), and the aforementioned physical barriers of the ocular surface epithelia, are just some features of the innate immune system at the ocular surface.[23] These mechanisms work together to prevent the negative outcomes of organism colonization in an effort to keep the ocular surface free from infection. However, if these mechanisms are overwhelmed and/or can be circumvented, then an organism can take hold, requiring activation and participation of the adaptive immune system for effective elimination of the pathogen.

Adaptive immunity results from an encounter with a specific antigen (eg, microbial proteins, allergens) and is therefore acquired through experience.[24] The adaptive system, which demonstrates memory for future encounters as well as specificity to antigens, is triggered by innate antigen-presenting cells, such as dendritic cells, and involves activity of various subsets of T lymphocytes and antibody-producing B lymphocytes, which facilitate pathogen removal and disease resolution. It should be noted that uncontrolled activity of the innate immune system can lead to a damaging inflammatory response, while erroneous activation of the adaptive system can result in autoimmune diseases. Indeed, a significant role for T lymphocytes in perpetuating dry eye inflammation itself is now recognized, and it appears that ocular surface epithelial cells secrete inflammatory molecules[17, 25] in response to cytokines produced by activated T lymphocytes.[26]

Alterations in the ocular surface armor (ie, disrupted surface and altered tear film) in dry eye would be expected to give pathogens a “foot in the door.” However, as discussed below, the concept that dry eye patients have an increased risk for corneal infection is not well supported in the literature. This suggests that dry eye patients may have an enhanced innate immune response, preventing an organism from taking advantage of the disrupted ocular surface and reducing the risk for infection. The objective of this article is to review and discuss the evidence for innate immune mechanisms that may play a significant role in protecting the ocular surface in dry eye patients. While the 2007 Dry Eye WorkShop (**DEWS**) report formulated a definition for dry eye,[1] there is still inconsistency in the literature as to how dry eye is defined in both population-based epidemiological studies (largely symptom-defined) and clinical trials (symptom and sign-defined). For the purposes of this review article, the terms “dry eye” and related “ocular surface disease (indicating dry eye)” were used, with the original author’s interpretation of appropriateness in using the term.

II. Dry Eye, Ocular Surface Inflammation and Corneal Infection

This section addresses evidence from published studies for an association between dry eye disease and ocular surface infection. The influence of contact lens wear is also discussed, as it is well recognized that this modality of refractive error correction is linked to both corneal infection and dry eye. Finally, reported changes in the ocular surface microbiome in dry eye are described, as they are pertinent to understanding a possible association between dry eye and infection.

A. Infection Risk in Dry Eye Patient Populations

1. Dry Eye Population in General—An association between dry eye disease and microbial keratitis is often cited in publications, including the DEWS report,[27] textbooks,[28] and the popular optometric/ophthalmic press. Also, in informal discussions among eye care practitioners, it is often concluded that dry eye patients are likely to have an increased risk for infection. The rationale for the association between dry eye and microbial keratitis is largely two-fold. First, dry eye disease can be associated with a quantitative reduction or qualitative alteration in the tear film, thereby causing a possible decrease in protective tear proteins.[29] Changes can occur in various protective molecules (see section II. B); while some are indeed decreased, others are actually increased at the ocular surface. Secondly, dry eye is often associated with a disruption of the corneal epithelium, thus creating a possible opening for microbial invasion.[30, 31]

Clinically, varying degrees of fluorescein staining of the cornea and conjunctiva are seen in dry eye patients.[32] While it is accepted that fluorescein staining indicates some level of compromise to the ocular surface epithelia, there continues to be much debate regarding the underlying etiology for the staining. Studies have suggested that loss of tight junction integrity allows deeper penetration and pooling of fluorescein between cells or that the dye is staining dead or damaged cells.[33–35] A recent study by Mokhtarzadeh et al suggests that the punctate epithelial staining characteristic of ocular surface disease such as dry eye results from enhanced fluorescence by some epithelial cells in the superficial layers.[36] The authors further speculate that these cells are likely interacting differently with fluorescein, perhaps because they are apoptotic or have lost the protective mucin barrier, a component of innate immunity.[36] Fluorescein staining does not necessarily correlate with susceptibility to infection. This has been shown in studies on contact-lens related microbial keratitis[37] and in mice deficient in MyD88, an adaptor molecule involved in the innate immune response.[38] However, irrespective of fluorescein staining and its interpretation, studies in murine experimental dry eye and human patients indicate a disrupted ocular surface and, in severe cases, epithelial sloughing, ie, a compromise to the normal protective epithelial barrier.

Despite the controversy regarding the nature of fluorescein staining, the procedure continues to be a mainstay in the diagnosis of dry eye and is often considered one of the key features in ocular surface and lid disease.[39,40] However, it is not clear whether the changes seen in staining patterns are part of the dry eye ocular surface disease process, or are a part of the inflammatory response, or a combination of both. Overall, very little literature exists to support the hypothesis that dry eye patients demonstrate an increased risk for microbial keratitis; however, it should be acknowledged that the diagnosis of microbial keratitis in patients with dry eye may well be under-reported. The few peer-reviewed published studies reporting an association between dry eye and microbial keratitis are described below.

A 2009 study of patients residing in nursing homes found that 26% of microbial keratitis cases, with *Staphylococcus* being the most prevalent isolate, were associated with the presence of dry eyes.[41] However, most patients in this study suffered from rheumatoid arthritis (**RA**) and were concurrently using topical and/or systemic steroid therapy, which

could increase the risk for infection, as well as alter the dry eye status.[41] A case of mycobacterium keratitis was reported in a patient with Sjögren syndrome (**SS**)-related dry eye, but, notably, this patient also suffered from RA.[42] It has been shown that RA is associated with dry eye disease, yet the link between RA and increased risk of infection in dry eye disease has not been established.

Boiko et al found an increased prevalence of dry eye in patients testing positive for Chlamydia conjunctivitis.[43] However, Krasny et al noted that patients who were successfully treated for chronic follicular conjunctivitis due to Chlamydia infection demonstrated improvement in their dry eye condition,[44] suggesting that, in some instances, ocular surface infection can predispose to dry eye rather than the dry eye predisposing a patient to infections. Several studies indicate that patients with dry eye-associated systemic autoimmune diseases, such as SS, RA, and ocular cicatricial pemphigoid, have an increased risk for sterile, but not infectious, corneal ulceration.[45–49]

Other studies also allude to a link between dry eye and microbial keratitis, but use the catch-all term “ocular surface disease,” making it difficult to determine true prevalence of dry eye in contrast to other “ocular surface diseases,” including infection and allergy. In one such study, ocular surface diseases such as herpetic corneal infection, bullous keratopathy, dry eye, blepharitis, and other eyelid disorders were shown to increase the risk for bacterial keratitis in 64 of 300 (21.3%) eyes (291 patients).[50] Indeed, a history of ocular surface disease was the second most common factor associated with bacterial keratitis in this study, with contact lens wear being the primary association and acute corneal trauma the third. Of the 64 patients in this “ocular surface diseases” subset,[50] 28 had pre-existing keratopathies (herpetic/bullous/exposure), while the remaining 36 patients had “other disorders,” including dry eye and eyelid diseases. If all of these 36 patients originally suffered from dry eye, that would mean a 12% (36/300) risk for dry eye to predispose for bacterial corneal infections.

Keay et al found in a retrospective review of medical records that 5.8% of patients presenting with microbial keratitis had ocular surface disease as a predisposing factor.[51] In a similar retrospective study for treatment of “keratitis,” Green et al found that a pre-existing history of ocular surface disease was present in 45 of 177 patients (18%) who had microbial keratitis.[52] In many of these studies, ocular surface disease was not specifically defined, but it was noted that these patients tended to have more severe keratitis and took longer to recover.[53] However, other factors may have contributed to the association between ocular surface disease and keratitis. In this same patient population,[52] the authors reported that contact lens wear (22% of the patients) was the most common risk factor for keratitis.

Overall, from the evidence in existing literature, it is apparent that in certain situations (coexisting systemic autoimmune disease/patients on corticosteroid therapy), dry eye patients may have a slightly increased risk for bacterial infections. Surprisingly, however, there is insufficient evidence to strongly suggest that a typical dry eye patient will have an increased risk for microbial keratitis. Indeed, in a recent preliminary study where *Pseudomonas aeruginosa* (*P. aeruginosa*) was topically inoculated in a murine model of dry eye, there was no visible pathology in either dry eye mice or controls.[54] The authors concluded that a dry eye state does not increase susceptibility to infection. It is possible that the animal model used was not ideal (no corneal staining was demonstrated), but the findings warrant further investigation.

2. Contact Lens Vs Non-Contact Lens Wearers—Although no firm evidence exists for an association between dry eye in general and an increased risk of microbial keratitis, can the same be said about contact lens wearers with dry eye? The risk of microbial infections in the soft contact lens-wearing population is well known and has been

extensively cited in the literature.[55–58] The reported incidence of microbial keratitis is equivalent regardless of soft contact lens material, varying from 5.2/10,000 people using daily wear soft contact lenses to 18.2/10,000 in those wearing reusable extended wear soft contact lenses.[59,60] Contrary to expectations, silicone hydrogel contact lenses with an increased level of oxygen transmissibility are not associated with a reduced rate of keratitis compared to traditional hydrogel contact lenses, with both materials having a similar risk.[61,62] As lack of oxygen does not appear to be the prime associated factor for eliciting microbial keratitis, other factors have been suggested, including impaired tear exchange, bacterial binding to contact lens material, decreased epithelial cell desquamation, and increased corneal permeability.[37,63–66] Although highly oxygen-permeable silicone hydrogel lens materials are considered a risk factor, especially in a closed-eye modality, the cases of microbial keratitis occurring with these lenses have been less severe than those reported in hydrogel lens wearing populations.[67]

The biggest risk factor, whether with silicone hydrogel or hydrogel lenses, continues to be the modality of continuous or extended wear, fundamentally a closed-lid scenario. This may relate to factors mentioned above or could result from the combination of tear film alteration by the contact lens and tear film changes that take place in a closed-eye situation.[68–71] Both appear to be independent factors that occur during lid closure; however, the relationship between the two is not as well defined. A contact lens, regardless of material, will alter the environment and therefore the ocular surface when worn on a regular basis. The cornea during contact lens wear will have a decrease in epithelial cell sloughing with a concomitant increase in epithelial cell size, as well as a decrease in epithelial thickness.[72–77] The alteration of the epithelial surface may alter normal apoptosis and allow for bacteria to enter the epithelium via lipid rafts.[78,79] Additionally, the post-lens tear film dynamics are altered in contact lens wear, which may affect receptor sites and allow for a potentially greater adherence of bacteria such as *P. aeruginosa*.[79,80] Lin et al have shown that in addition to hypoxia, tear stagnation in the post-lens tear film increases corneal epithelial permeability, which may put the cornea at a greater risk for infection.[81] To date, it has also been shown that sIgA, an important antimicrobial protein, decreases with closed-eye contact lens wear, and interleukin (**IL**)-8 increases with a lessened effect noted for both in silicone hydrogel lens wear.[82]

It is not known if the risk of microbial keratitis in contact lens wearers with dry eye is greater than in contact lens wearers without dry eye symptoms. Contact lens-related dry eye is a common complaint with manifest signs in a large proportion of these patients. Depending on the studied population, it has been reported that between 50% and 94% of contact lens wearers complain of dry eye symptoms,[83–88] often leading to decreased wearing time or discontinuation of contact lens wear.[89–92] Some studies have suggested that high-water-content soft contact lenses are associated with more pronounced symptoms of dry eye.[93] The reason for this has not been clearly elucidated, although it has been suspected that it is due to increased evaporation from this type of lens. However, data to support this claim is lacking.[89,94] Interestingly, while contact lens material can play a role in exacerbating the dry eye, it can also serve to ameliorate it, with studies showing a decreased occurrence of dry eye symptoms with silicone hydrogel contact lenses.[88,95–97] Recently, scleral gas-permeable contact lenses have been promoted as a therapeutic option for severe dry eye,[98,99] and increased occurrence of bacterial keratitis in patients using this therapeutic modality of lens wear for dry eye has not been reported.

Contact lenses can affect aqueous tear production by acting on the neurosensory loop. They can also be an extrinsic factor interrupting the normal patency of the tear film, creating an evaporative dry eye. Recent work has indicated that even silicone hydrogel lenses worn on a daily wear basis alter tear film stability and tear film components such as MUC5AC and

IL-6, which have antimicrobial actions.[100,101] As noted above, tear stagnation under a contact lens may put the cornea at a greater risk for infection.[81] It might be suspected that tear stagnation could occur to an even greater extent in the dry eye, hence presenting an increased risk, especially in the aqueous-deficient dry eye, ie, the available volume of tears is less, so the amount of fresh tear film exchanged under the contact lens with each blink is less.

In a recent study, Berry et al compared protein expression in contact lens wearers with and without dry eye.[102] Using several proteomic approaches, they found a number of potential biomarker proteins associated with the dry eye disease state in contact lens wearers. In particular, beta-2 microglobulin, proline rich 4, lacritin, and secretoglobin 1D1 were downregulated, while secretoglobin 2A2, serum albumin, glycoprotein 340, and prolactin-inducible protein were upregulated. The authors concluded that the functions of several of these proteins suggest roles in altered tear secretion in addition to possible increased susceptibility to infection.

Other factors associated with microbial keratitis include male gender, age, smoking status, humidity, and lens care system compliance and modality.[62,103–105] Most risk factor profiles appear to implicate young contact lens wearers and those who have just started wearing contact lenses, neither of which are patient groups that are particularly known to exhibit dry eye disease. As is evident from this discussion, despite the known risk of microbial keratitis with contact lens wear, a relative increased risk with contact lens-related dry eye is circumstantial at best and has not been specifically investigated in epidemiologic studies.

In summary, in peer-reviewed and non-peer-reviewed literature and presentations in public forums, dry eye is often cited as a predisposing factor for infectious keratitis. Although this is widely believed as logical and possibly credible, evidence linking dry eye and infectious keratitis is minimal for both the general dry eye population and those with contact lens-related dry eye. Specific epidemiological studies to determine the risk of infectious keratitis in dry eye would be informative but costly and time-consuming, and have not been completed to date. However, as population-based studies of keratitis continue to be reported, meta-analysis of published data or nested case control study designs may be feasible to better determine the risk for infection in dry eye patients.

B. Dry Eye and Ocular Surface Microbial Load

In a recent review, Miller and Iovieno noted that the normal ocular surface harbors a diverse group of microorganisms, with Gram-positive bacteria such as *Staphylococci* species being the primary commensals recovered from lids, conjunctiva, and tears.[106] A recent DNA sequencing-based study showed that the healthy human conjunctiva can have a wide variety of microbes, such as *Pseudomonas, Propionibacterium, Bradyrhizobium, Corynebacterium, Acinetobacter, Brevundimonas, Staphylococci, Aquabacterium, Sphingomonas, Streptococcus, Streptophyta and Methylobacterium*.[107] Further, the microbial community remains relatively stable unless interrupted by events such as contact lens wear, exposure to preservatives, and ocular surface disease.[106]

A number of studies have addressed the ocular surface microbial load and changes in dry eye patients. The earliest (1975) study estimating load did not reveal the presence of adenovirus (types 3, 7, 8 and 14) or herpes simplex in 50 patients.[108] However, in a more recent study, Robert et al found human herpes virus-6 (HHV-6) in the tear fluid of 2 of 28 dry eye patients.[109] Albeitz and Lenton reported that the ocular surface of dry eye patients had a greater bacterial load compared to healthy patients and that SS patients had a greater bacterial load (*Cornyebacterium* species and *Propionibacterium* species) than non-SS dry eye patients.[110] Similarly, Graham et al reported greater loads of coagulase negative

staphylococci (normal flora at the ocular surface) in dry eye patients. They found certain common ocular surface bacteria (*Cornyebacterium* and *Propionibacterium)* as well as uncommon ones (*Rhodococcus erythropolis*, *Klebsiella oxytoca* and *Erwinia species)* to be present in normal patients and dry eye patients.[111] They concluded that the presence of these microbial pathogens at the ocular surface of normal and dry eye patients presented a "diagnostic dilemma": since the pathogens were present in all patient groups, antimicrobial therapy could not be justified for one group (dry eye patients).

Previous studies have shown that conditions that cause dry eye (such as anterior blepharitis, meibomian gland dysfunction, and ocular rosacea) are associated with a variety of bacteria such as coagulase negative staphylococci,[112–114] *Staphylococcus aureus* (*S. aureus*),[115] *Streptococcus species*, *Bacillus subtilis*,[116,117] *Rhodococcus* species,[118] *P. aeruginosa*,[119] and *Hemophilus influenza*.[116,117] Hori et al found no significant difference in bacterial isolation rates from conjunctiva of normal and dry eye patients, although the dry eye patients (SS and non-SS) had an increased presence of fluoroquinolone-resistant bacterial strains.[120]

It is not clear that any changes in ocular surface flora in dry eye are the result of the altered environment or if an altered flora may contribute to the disease process. Bacterial lipases and bacteriocins[121] produced by commensals at the ocular surface have been shown to damage cells of the surface and destabilize the lipid layer,[111] induce desiccating stress,[26] and cause loss of goblet cells,[122,123] indicating that bacterial action may exacerbate dry eye disease. Topical (tobramycin, azithromycin) and oral (doxycycline, minocycline) antibiotics alone or in combination with steroids such as dexamethasone or loteprednol have been shown to be beneficial in the treatment of blepharitis, which causes a secondary dry eye.[124–128] It has been hypothesized that these topical medications alter the normal eyelid and ocular surface flora, reduce inflammation, and possibly contribute to antibiotic resistance.[120,129,130] Some data suggest that drugs such as oral minocycline aid the treatment of blepharitis by decreasing or eliminating ocular surface flora.[128] Other data suggest that oral minocycline also inhibits lipases in blepharitis patients,[127] while oral doxycycline inhibits matrix metalloproteinase (**MMP**)-9 and thus helps blepharitic conditions.[125] A recently published case report of a methicillin-resistant *S. aureus* keratitis in a dry eye patient with a therapeutic contact lens highlights the complicated and multifactorial nature of ocular surface conditions and the difficulty in determining causative factors.[131]

Based on the limited evidence available, it appears that dry eye patients do tend to have an altered/greater microbial load at the ocular surface compared to healthy patients. However, greater prevalence of infections from these microbes does not seem to exist in dry eye patients. This suggests that dry eye patients may not be at a higher risk for infection, because innate immunity (and perhaps other mechanisms) adequately protects the ocular surface despite the presence of dry eye disease.

III. Dry Eye, Innate Immune System and Endogenous Antimicrobials

A. Ocular Surface Innate Defense Mechanisms

A comprehensive review of ocular surface innate defense mechanisms is beyond the scope of this article; suffice to say the ocular surface is well equipped for defense against pathogens through the physical barrier presented by the epithelia, the sloughing of epithelial cells, sensory nerves to trigger tearing/blinking, and a myriad of molecules, large and small, with direct or indirect antimicrobial properties. The latter arise from a number of cellular sources, including the ocular surface epithelial cells and glands involved in tear production. Some are derived from neutrophils that can be found in the tears on awakening. Figure 1 summarizes the major defense mechanisms. For more detail, the reader is referred to recent

review articles by Gregory[132] and McDermott.[133] Current literature on changes in innate defense mechanisms reported to occur with dry eye are reviewed below. The major findings are also summarized in Table 1.

B. Pathogen Recognition in Dry Eye

Sensing of pathogens via pattern recognition receptors (**PRR**s) is the primary way host cells detect the presence of unwanted invaders. The major PRRs are toll-like receptors (**TLR**s) and NOD-like receptors (**NLR**s), and these generally recognize specific pathogen-derived ligands referred to as *pathogen-associated molecular patterns*. Ocular surface epithelial cells and corneal epithelial-associated Langerhans cells are known to express a range of both TLRs and NLRs and thus can readily detect and respond to invading organisms via production of chemokines, cytokines, and antimicrobial peptides.[134,135] Enhanced expression of ocular surface PRRs may confer greater pathogen-sensing capabilities to the dry eye, contributing to a reduced risk of overt infection. However, it may also be a source of inflammation, as TLR activation stimulates the production of many proinflammatory cytokines and chemokines found in dry eye.

Some preliminary studies have addressed modulation of TLR expression in vitro and in vivo. Culture conditions mimicking a dry eye environment upregulated ocular surface epithelial cell expression of TLR4 and 5, which detect bacterial lipopolysaccharide and flagellin respectively, but downregulated TLR9, an endosomal receptor that detects unmethylated CpG motifs on bacterial and viral DNA (Redfern and McDermott [unpublished data]). In conjunctival epithelia of patients with dry eye disease, TLR2 (which detects bacterial lipopeptides) mRNA but not protein was upregulated,[136] TLR4 was increased (although not significantly so) and TLR9 was downregulated.[137]

In a recent study using an experimental dry eye mouse model, Lee et al found that corneal epithelial and stromal TLR4 expression was increased, and that TLR4 inhibition decreased the severity of dry eye corneal staining and significantly reduced cytokine expression and infiltration of immune cells into the cornea and lymph node.[138] In the same mouse model, we have observed upregulation of TLR2-4 and 9 in conjunctiva, cornea, and/or lacrimal gland.[137] These investigations show that TLR expression is modulated at the ocular surface in dry eye conditions. They are corroborated by other studies showing enhanced TLR expression in labial salivary glands of human SS patients, a form of dry eye with an autoimmune basis, and SS mouse models.[135] However, the significance of modulated TLR expression in dry eye and its influence on risk of ocular surface infection in dry eye has yet to be elucidated. Further NLRs and the complement system, which is constitutively active at low levels in the tears and may be viewed as a pathogen detection system, have not yet been investigated in dry eye.

C. Lactoferrin, Lysozyme, Lipocalin, sIgA, and Phospholipase A2 in Dry Eye

The levels of lactoferrin and lysozyme in human tear fluid have been of great interest to dry eye researchers for over three decades. Lactoferrin is secreted by acinar cells of the lacrimal gland.[139] Lactoferrin binds iron in tear fluid, and, thus, bacteria are unable to colonize the ocular surface due to the lack of this nutrient.[140,141] Lactoferrin is known to have anti-inflammatory, anticancer, and immune-modulating properties, in addition to possessing antimicrobial activity against a wide range of microbes (bacteria, fungi and viruses).[142] Lysozyme, which attacks cell walls of bacteria,[141] is secreted by the main lacrimal gland acinar cells[143] and conjunctival accessory lacrimal glands.[144] Several studies have demonstrated that the levels of lysozyme and lactoferrin are decreased in the tear fluid of SS and non-SS dry eye patients compared to healthy patients. [114,145–149]

Two recent studies have reported the levels of lipocalin in the tear fluid of dry eye patients.[150,151] Lipocalin, which is secreted by the main lacrimal gland acinar cells,[143] produces bacteriostatic activity by binding to bacterial ferric siderophores and preventing siderophore-mediated iron uptake by bacteria. Caffery et al showed that lipocalin is reduced in SS patients compared to non-SS dry eye and healthy patients.[150] They also demonstrated that lipocalin levels are not different between non-SS dry eye patients and healthy humans. Versura et al showed decreased levels of lipocalin and lactoferrin in the tear fluid of evaporative dry eye patients.[151] However, both these studies demonstrated no difference in lysozyme levels in the tear fluid of SS, non-SS, or healthy patients.[150,151]

Secretory IgA (**sIgA**) plays a major role in antimicrobial protection of mucosal tissue.[152] Plasma cells in the main lacrimal gland as well as in the conjunctival accessory lacrimal glands produce immunoglobulin-A (**IgA**).[153,154] When excreted in to the tear film, a protein referred to as *secretory component* is associated with IgA dimers. SIgA may modulate the normal flora at the ocular surface and thus provide protection to the surface. In the intestine, sIgA uses “immune exclusion” to clear antigens and pathogens by either preventing access to epithelial receptors or by capturing them in mucus and promoting their removal by peristalsis.[152] Seal et al showed that tear fluid sIgA levels were decreased in 3 of 12 eyes (6 dry eye patients), and sIgA levels were in the low normal ranges for the other eyes.[114] Boukes et al found a decrease in sIgA in the tear fluid of SS patients compared to healthy controls,[145] while Wehmeyer et al did not find any difference between these two groups of patients.[155]

sIgA might play an important role in preventing bacterial adhesion on contact lenses.[156] Contact lens-wear decreases the levels of sIgA in tear fluid,[157] and extended wear of contact lenses, which is associated with a greater risk of corneal infections, also decreases tear concentrations of sIgA epitopes.[158] It can be hypothesized that decreased levels of sIgA inhibits proper modulation of ocular surface flora, whereby pathogenic/opportunistic organisms colonize the ocular surface. This situation can arguably increase the risk for microbial infections at the ocular surface.

Secreted Phospholipase A2 (**sPLA2**) is a pro-inflammatory enzyme that catalyzes the initial step of the arachidonic acid pathway.[159] sPLA2 binds to the anionic bacterial surface due to its cationic nature and kills via its phospholipolytic enzymatic activity.[160,161] Group II sPLA2 in the tear fluid plays a major role in killing a broad spectrum of Gram-positive bacteria at the ocular surface under physiologyical conditions.[162] sPLA2 can also kill Gram-negative bacteria with the help of additional antibacterial compounds, such as the bactericidal/permeability-increasing protein.[160,161] The ocular surface has increased levels of sPLA2 in tear fluid of dry eye patients,[163] increased activity in tear fluid in dry eye patients,[164] and increased expression of mRNA and protein in dry eye mouse conjunctival tissue.[165] The mouse study did not detect sPLA2 in the normal or dry eye corneas and noted that a sPLA2 inhibitor reduced the level of sPLA-2-induced inflammation in this model.[165]

From the above discussion, it is very clear that sPLA2 plays a major role in the prevention of microbial infections at the ocular surface in dry eye conditions.

D. Mucins in Dry Eye

Mucins are high molecular-weight proteins, with tandem repetitions in the central portion of the molecule.[166] The ocular surface expresses at least 9 of the 18 known human mucin genes: MUC1, 2, 4, 5AC, 7, 13, 15, 16, and 17.[167–178] Mucins keep the ocular surface wet and protected from adverse environmental conditions. Based on their amino acid sequences, mucins are categorized in three distinct families: gel forming (MUC2, 5AC, 5B, 6, and 19), soluble (MUC7 and MUC9), and transmembrane (MUC1, 3A, 3B, 4, 12, 13, 15–17, 20, and

21); other mucins remain unclassified (MUC8 and MUC11). MUC1 and MUC16 exist as membrane-bound mucins on ocular surface epithelia as well as soluble mucins in the tear fluid.[179] The membrane-bound form of MUC1 clears bacteria from the ocular surface by binding the bacteria and is later cleared out of the surface as a bacterial-mucin complex.[179] MUC16 helps prevent bacterial adhesion.[180] It has been shown previously that the frequency of non-SS aqueous-deficient dry eye patients expressing only the MUC1/A splice variant of the mucin MUC1 may be lower than that of a normal control group.[181] Thus, a longer repeat sequence of amino acids on MUC1 variants may play role in susceptibility to dry eye syndrome, as they provide better quality of lubrication and protect the surface from inflammation.[181]

Differences in MUC1 genotype can explain the loss of ocular surface integrity that is often observed in dry eye patients.[12,16] Blalock et al demonstrated that molecules such as neutrophil elastase and tumor necrosis factor-alpha (**TNF-α**) promote the release of MUC1, MUC4, and MUC 16 in human corneal-limbal epithelial cells.[182] Similar release of membrane-associated mucins can be induced in dry eye patients who often demonstrate increased levels of inflammatory cytokines, such as TNF-α.[183] The release of these mucins from the corneal epithelial surface can lead to loss of integrity of the ocular surface in dry eye patients (evidenced in clinical practice as vital dye positive staining), which can open pathways for pathogens to invade the cornea. Interestingly, Caffery et al found an increase of soluble MUC1 in SS patients and non-SS patients compared to healthy patients.[184] They also found MUC1 mRNA expression to be similar in non-SS and healthy patients, while membrane-bound MUC1 expression was different only between the SS and healthy patients.[184] The difference in results for MUC1 expression shown in this study could be attributed to factors such as pooling impression cytology samples, which can mask individual patient variability.[184] The authors suggested that the differences in MUC1 expression between the three groups of patients indicates a preventative response from the ocular surface to avoid infections/inflammation.[184]

Other recent studies have demonstrated a decrease in MUC5AC (mRNA and protein), altered distribution of MUC16 epitopes, and decrease in MUC19 (mRNA and protein) levels in SS patients compared to healthy patients.[167,179, 185–187] The chemical composition of gel-forming mucins such as MUC5AC can be altered by bacterial ligands, such as lipoteichoic acid (Gram-positive), flagellin A, and LPS (Gram-negative).[188] Alteration of these gel-forming mucins can cause epithelial stress and thereby activate the adaptive immune system, resulting in T lymphocyte infiltration, cytokine secretion, and death of surface epithelial/goblet cells.[188] Mucins can be thought of as providing a physical barrier to the entry of bacteria.[189] As described earlier, the presence of MUC16 at the ocular surface provides a nonadhesive barrier to prevent bacterial entry.[180]

In summary, loss of ocular surface mucins may result in loss of a physical barrier against pathogens, loss of corneal integrity (vital dye staining), and epithelial stress that activates the adaptive immune system, causing T lymphocyte infiltration and cytokine secretion (leading to ocular surface epithelial cell death). Thus, it can be argued that the alteration of ocular surface mucins in dry eye states places the eye at risk for microbial infections.

E. Antimicrobial Peptides and Dry Eye

Antimicrobial peptides (**AMP**s) such as defensins and cathelicidins, are important innate defense molecules at the ocular surface.[190–200] We have shown that the ocular surface epithelia constitutively express human β-defensin (**hBD**)-1 and hBD-3, while hBD-2 expression is observed only in response to inflammatory cytokines, infections, and injury.[190,192–196,199–201] We have also shown that enhanced expression of the cathelicidin LL-37 is observed at the ocular surface in response to inflammatory cytokines and

infection.[190,191,197,202] Low levels of α-defensins (human neutrophil peptides [HNP])-1, -2 and -3 have been detected in the tear fluid, with the primary source being neutrophils that can be found in the tears upon eye opening.[203] hBD-2 and -3 have also been detected in low levels in reflex tears and basal tears (hBD-2 only).[204] The origin of these tear β-defensins is not known for certain, but it may be ocular surface epithelial cells and/or lacrimal gland.

In terms of antimicrobial effectiveness, hBD-1 is not significantly effective against the common ocular pathogens *P. aeruginosa* or *S. aureus*.[191,205] However, hBD-2 has good activity against *P. aeruginosa*, while hBD-3 and LL-37 have good activity against both *P. aeruginosa* and *S. aureus*. Several studies using murine models have shown the importance of defensins and the murine cathelicidin cathelin-related antimicrobial peptide (**CRAMP**), the ortholog of human LL-37, in protection against *P. aeruginosa* keratitis. A recent study with stratified cultured human corneal epithelial cells showed that defensins were essential to prevent traversal of the bacteria across the epithelial barrier.[202,206,207]

We found that dry eye patients demonstrate an upregulated expression of hBD-2 compared to healthy patients, which may be mediated by enhanced pro-inflammatory cytokine expression.[137,200,201] However, there was no difference in the expression of hBD-1 and -3 between the two groups of patients.[201] Later, Huang et al, in another laboratory, confirmed increased conjunctival expression of hBD-2 in SS dry eye patients as well.[208] It should be noted, however, that salivary gland expression of hBD-1 and -2 has been shown to be decreased in SS patients.[209] We found that conjunctival expression of LL-37 does not change in dry eye patients compared to healthy patients,[137] and Abedin et al reported decreased corneal and conjunctival expression of hBD-9 in dry eye.[210] Thus, these data indicate that the dry eye retains significant protection through hBD-1, hBD-3 and LL-37, the levels of which do not appear to be decreased compared to normal, and that additional protection may arise from the enhanced expression of hBD-2. The decrease in hBD-9 is not expected to be of significance, as this particular defensin is not predicted to have potent antimicrobial activity.[210]

It should be noted that the antimicrobial action of some AMPs (eg, hBD-1 and -2) is salt-sensitive and is compromised by salt and mucins in the tear film,[191,192,205] calling into question their role as antimicrobial agents at the ocular surface. However, at least for hBD-2, the elevated levels in dry eye may go some way to compensate for these detrimental effects.[191] We have performed a limited investigation of AMP expression in a dry eye mouse model and found decreased corneal and/or conjunctival expression of mBD-1 and CRAMP but increased or no change in expression of mBD-3 and -4 (the latter are orthologs of human hBD-2)[17,137].

Although more study is required, these early data mostly show similar trends to changes in AMP expression in human dry eye; thus, the murine dry eye model may be useful for investigating the functional consequences of modulated AMP expression in vivo.

While AMPs are known to have direct antimicrobial activity, they also exhibit a variety of immunomodulatory behaviors and modulate wound healing. Thus, their protective role in dry eye may be not only to effect direct pathogen killing, but they may also, for example, influence immune cell actions, thus indirectly maintaining and enhancing antimicrobial protection. AMP mechanisms of antimicrobial action and their plethora of other actions are the subject of several recent reviews.[141,211–213]

F. Modulation of Other Defense Mechanisms

Some patients with dry eye disease frequently experience significant epiphora[214] as a feedback response to an irritated and dry ocular surface. This response serves to bathe the

ocular surface with replenished tear film and help rid it of irritants or foreign objects. Might this same response also protect the patient with dry eye disease from infection by decreasing the microbial load on the ocular surface? No studies examining this have been performed. However, it has been shown that SS patients having decreased salivary flow rates exhibited a higher load of *Candida albicans*.[215] Since the oral cavity does not benefit from the same compensatory mechanism as the eye, it might be inferred that an increased rate of tear fluid production would serve to decrease certain microbes. Interestingly, dry eye patients demonstrate decreased corneal sensitivity[216] and reduced blink efficiency.[217] Therefore, the mechanism for the increased reflex epiphora in dry eye patients seems unclear.

The basement membrane of the corneal epithelium is known to provide an important barrier to pathogen entry into the stroma, although this can be compromised, for example, by bacterial proteases.[218] The dry eye environment enhances production of degradative MMPs, and an increased risk of corneal ulceration has been reported in severe cases of dry eye.[34,219,220] These data suggest the possibility of basement membrane compromise in dry eye, although direct evidence for this in the literature is lacking.

In summary, there is evidence to support reduction in some ocular surface antimicrobial molecules and possible compromise of other defense mechanisms. However, to balance this, important chemical defense molecules, such as sPLA2 and AMPs, are either unchanged or enhanced. Further, in addition to the molecules discussed above, there are a number of other ocular surface molecules with known antimicrobial properties, such as surfactant protein D.[221] How their expression is modulated, if at all, in dry eye is not yet known, but they may possibly also be increased, so contributing to enhanced ocular surface protection. Thus, we propose that in dry eye, it is possible that the enhancement of innate immune molecules as described above serves to effectively clear any pathogens attempting to invade, preventing them from reaching and causing damage to or taking advantage of a possibly compromised basement membrane to gain entry to the stroma. In contrast, contact lens wear appears to reduce the ability of the ocular surface to respond to pathogens. For example, a reduced ability of *P. aeruginosa* to induce hBD-2 expression was noted in an in vitro model of contact lens wear, leaving the cornea with a reduced capacity to clear pathogens.[222] Hence, the disrupted epithelial surface of the contact lens wearer is likely at greater risk of infection than the dry eye-disrupted epithelium. An important comparison yet to be investigated is the state of innate defenses in contact-lens wearing patients who also have dry eye.

IV. Dry Eye Treatment and Modulation of Risk for Keratitis

Treatment of dry eye is often aimed at replenishing the tear film, increasing tear retention, or dampening inflammation on the ocular surface. The palliative and therapeutic agents used for these purposes may also contribute to limiting the microbial load on the ocular surface by simply washing out and/or diluting invading pathogens or by altering the ocular surface physiology to increase microbial killing or decrease their capacity to invade. Common dry eye treatment modalities and their potential impact in modulating the risk for microbial keratitis are discussed below.

A. Artificial Tears

Artificial tears are often one of the first treatment options given by eye care practitioners to dry eye patients,[223] and they are sometimes recommended to patients with microbial keratitis to relieve ocular surface irritation. A variety of artificial tears are available over-the-counter with a plethora of formulations.[224] Benzalkonium chloride (**BAK**) is the most commonly used preservative in ophthalmic products, largely due to its proven antimicrobial efficacy.[225] BAK, at a low concentration (0.005%) is able to kill *S. aureus* and coagulase-negative *Staphylococcus*,[226,227] but not *P. aeruginosa*.[227] These data suggest that in addition

to replenishing the tear film, artificial tears may also reduce the risk of staphylococcal infection in dry eye patients. However, preservative-free artificial tears are more commonly being recommended for frequent use to replenish the tear film, as BAK can be cytotoxic to human corneal epithelial cells,[205] thus nullifying their potential antimicrobial effect. Further, although implicated in reducing microbial loads, topical artificial tears may increase corneal epithelial permeability,[228] perhaps leading to increased corneal staining clinically. As previously mentioned, increased corneal permeability may allow microbial penetration into the cornea,[19, 229] although it has been suggested that, for this to occur, additional risk factors need to be present.[63]

As mentioned in section III. E, the ocular surface epithelial cells produce antimicrobial peptides, including hBD-2 and LL-37, which are capable of killing a variety of pathogens.[191,192,202] However, it has been observed that both hBD-2 and LL-37 lose their ability to kill *P. aeruginosa* in the presence of carboxymethylcellulose-containing artificial tears in vitro, possibly adding to apparent detrimental effects of artificial tear solutions on ocular surface immunity.[230] Clinically, we often observe that dry eye patients use artificial tear solutions several times per day, which may itself contribute to the flushing out of pathogenic microbes.

B. Cyclosporine

It is well documented that inflammation is a key component in the pathogenesis of dry eye. This is borne out by the efficacy of anti-inflammatory agents, such as corticosteroids (discussed in section IV. C) and cyclosporine for treatment of dry eye disease.[231] Topical cyclosporine significantly alleviates the signs and symptoms of dry eye[232] and is often prescribed for long-term use by eye care practitioners. Topical steroids have also been used to treat more severe forms of dry eye disease, often in combination with cyclosporine at the initiation of dry eye therapy. Cyclosporine selectively inhibits T lymphocyte-dependent production of proinflammatory cytokines involved in a myriad of ocular surface inflammatory conditions. It exerts its effects by forming a complex with cyclophilin, which binds and inhibits calcineurin,[233] preventing the translocation of nuclear factor of activated T cells, a family of transcription factors, from the cytoplasm to the nucleus. This interaction ultimately inhibits the production of proinflammatory cytokines, therefore dampening the immune response on the ocular surface.[234]

In addition to reducing the production of proinflammatory cytokines in dry eye disease,[235] cyclosporine is also thought to dampen the expression of immune activation markers (HLA-DR and CD40) by conjunctival epithelial cells[236] and decrease CD3, CD4, and CD8 positive-T lymphocytes in the conjunctiva of dry eye patients.[237] Guzey et al showed that in patients with trachomatous dry eye, central corneal thickness increased with cyclosporine treatment.[238] They attributed this to an improvement in the integrity of the ocular surface and resolution of the underlying inflammation. However, cyclosporine may have adverse side effects, especially when used chronically. With the reduction in lymphocytes and the compromised ocular surface, does the use of cyclosporine in dry eye disease increase the risk of microbial keratitis? Does cyclosporine's effect on the inflammatory cascade coincide with an alteration of the localized immune response on the ocular surface?

In an in vitro study, Hara et al showed that cyclosporine may increase the susceptibility of human corneal epithelial cells to viral infection by reducing the production of IL-6, IL-8 and NF-kB activation in response to TLR3 activation by pathogen-associated molecular pattern sequences.[239] As discussed below, several studies have examined the risk for infection with topical cyclosporine use in human dry eye patients and animals.

In patients with concurrent herpes simplex keratitis (**HSK**) and dry eye disease, a 1-year treatment with cyclosporine actually reduced the duration of HSK recurrences rather than increasing the risk of infection.[240] In a multicenter, double-masked study, Stevenson et al investigated the safety of twice-daily dosing topical cyclosporine treatment at doses ranging from 0.05% up to 0.4% for 12 weeks in patients with moderate-to-severe dry eye.[241] This study found no significant adverse effects, no microbial overgrowth on the ocular surface, and no increased risk of ocular infection in any of the cyclosporine-treatment groups. Similarly, in an in vivo study on the effect of topical cyclosporine on microbial colonization of the corneal surface of dogs with dry eye, Salisbury et al found that in dogs that responded to cyclosporine treatment, as indicated by a significant increase in tear production, the percentage of eyes from which bacteria were isolated after 3, 6, and 12 months of cyclosporine treatment was significantly less than it was prior to treatment.[242] They also found that the percentage of eyes from which fungi were isolated decreased during the course of cyclosporine treatment. Consistent with this, cyclosporine treatment has been shown to have a significant suppressive effect on the growth of *Fusarium oxysporum* and *Fusarium solani*, compared to vehicle or methylprednisolone treatment[243] and can also have a synergistic effect with the antifungal medication fluconazole in cases of fungal keratitis.[244] Together, these studies suggest that use of cyclosporine is not associated with an increased risk of ocular surface infection.

In severe dry eye disease, the ocular surface may become compromised, possibly leading to ulceration, ultimately initiating a wound healing response. Cyclosporine has been shown to inhibit the production of cytokines and chemokines involved in wound healing,[239,245] and studies have investigated the effect of cyclosporine on modulating the immune response when the ocular surface is compromised. Flueckiger et al used an ex vivo, whole-globe porcine model to investigate corneoepithelial wound healing in response to cyclosporine, and found that cyclosporine had no influence on corneoepithelial wound healing.[246] However, Garweg et al, in a study of human conjunctival epithelial cells, found that cyclosporine inhibited cell proliferation, with a corresponding decline in cell viability, as detected by a decrease in calcein metabolism.[247]

Previous studies have shown that wound healing and dry eye increase the expression of antimicrobial peptide, hBD-2 in ocular surface cells,[137,200,201] suggesting that hBD-2 may provide additional antimicrobial protection when the ocular surface is compromised. Interestingly, cyclosporine has been shown to downregulate hBD-2 expression in human corneal epithelial cells in vitro,[248] and, therefore, the same scenario may exist when cyclosporine is used in patients to treat dry eye inflammation. A potential downregulation in hBD-2 could increase the risk for infection in dry eye patients. This change may not be physiologically relevant in dry eye, as hBD-2 may lose some of its antimicrobial effectiveness on ocular surface of dry eye patients due to hyperosmolar stress and potential interactions with mucins in the tear film.[192,195]

C. Steroids

In addition to cyclosporine, other immunomodulatory drugs such as steroids are often used to dampen inflammation on the ocular surface in dry eye. The effect of corticosteroids on the inflammatory cascade, specifically the blockade of cyclooxygenase and production of prostanoids from arachidonic acid, is well known and is likely the reason this form of therapy has been efficacious in practice. Corticosteroids also exert local immunomodulatory activity through the inhibition of certain transcription factor activity.[223]

An increased susceptibility to infection with the use of topical steroids has been cited in the literature.[249] This has been documented with the case of susceptibility to fungal infections.[249,250] However, Ilyas et al reported no significant corneal adverse events,

including corneal infections, in a subject population using loteprednol etabonate 0.2% for allergic conjunctivitis for a minimum of 1 year.[251] This could in part be due to the specific design of loteprednol, which replaces the typical ketone group in other steroids with a 17α-chloromethyl ester.[252,253]

Recently, Suto et al examined the bacteria isolated from the conjunctival sac in pre-cataract surgery patients with and without dry eye, who either were taking oral steroids or using artificial tears or other topical medications (steroid).[254] The bacterial isolation rate was significantly lower in dry eye patients (using only artificial tears) than for those without dry eye disease, and the use of artificial tears reduced the bacterial isolation rate compared to patients not using topical medication. Patients taking oral steroids did not have a significant difference in bacterial isolation rates. Similar results were found in dry eye patients taking topical steroids. This study reported that topical steroid use by dry eye patients did not alter the bacterial profile on the ocular surface compared to dry eye patients treated with punctal plugs or artificial tears.

These studies suggest that topical steroids are effective at reducing ocular surface inflammation in dry eye while not increasing the risk for infection.

D. Antibiotics

Macrolide antibiotics (azithromycin) and tetracycline derivatives (tetracycline, doxycycline, and minocycline) have been commonly used in the management of ocular surface and dermatological conditions, including anterior and posterior blepharitis, meibomian gland dysfunction, and rosacea. As a class, tetracyclines and their synthetic counterparts have been a frequently used therapy for the underlying causes of dry eye disease, and are thought to decrease inflammation and normalize production by the meibomian glands, in addition to having an antibacterial effect.[255,256] Their action in reducing MMPs[257] has also been useful in ameliorating the effects of inflammation after corneal ulcer treatment.[258]

Tetracyclines also serve to decrease the production of inflammatory cytokines IL-1α and TNF-α.[256,259,260] Although tetracyclines are broad spectrum antibiotics effective against Gram-positive and Gram-negative bacterial species, their effective prophylaxis against microbial infections in patients with dry eye has not been studied. Additionally, only minocycline and doxycycline reach levels that would be considered beneficial as an antimicrobial at the tear film ocular surface interface.[261] This was shown most recently in a small clinical study that demonstrated a decrease in ocular surface bacterial flora in patients who were started on 50 mg minocycline daily for 2 weeks, then switched to 100 mg daily for a total of 3 months.[127] At high concentrations, the tetracycline class of medications has been shown to have an inhibitory decrease in *Staphylococcal* exotoxin-elicited increases in cytokines and chemokines.[262] Tetracyclines may also act upon bacterial lipases with a resultant decrease in free fatty acids in the tear film.[263] However, to date, little is known about the relative proportion of the beneficial mechanisms involved in tetracycline therapy.

Topical azithromycin has been prescribed for use off-indication, alone or in combination with oral tetracycline derivatives, in the treatment of meibomian gland dysfunction and blepharitis, which often coexist with dry eye disease.[264] Studies to date have focused on the effectivity in reducing signs and symptoms of the disease, and have not reported a negative impact or evaluated the effect of chronic use on the normal microbiome and defense mechanisms of the ocular surface.[265–267] While no cases of ocular infection have been reported in these studies, further work is warranted to investigate the impact of preservative BAK as well as the chronic use of a topical antibiotic.

E. Future Studies

In the clinical management of dry eye patients, little thought is given to the potential negative effects of a systemic or topical therapy on the innate and adaptive immune systems of the ocular surface, although each therapy has the opportunity to directly or indirectly impact ocular surface immunity. Existing data do not seem to indicate that topical and oral therapies significantly reduce the ability of the ocular surface to respond to microbial threat, but further studies, including surveillance and natural history studies, may help to further elucidate the impact of chronic topical therapies on the ocular surface. In addition, techniques to accurately evaluate innate and acquired immunity in a clinical setting (or for clinical trials) are yet to be developed. The existing platforms of micro-tear collection used by the RPS viral detector as well as the TearLab osmolarity systems are technological advances that could be applied to further “biomarker” tear analysis. A detectable tear marker for increased bacterial load, for example, would be welcome in further understanding of the complex mechanisms at play on the ocular surface, with and without disease prior to and during treatment.

V. Summary and Conclusions

This article provides a comprehensive review of the current evidence, circumstantial and otherwise, for a link between microbial keratitis and dry eye. Although such a link is widely believed as logical and possibly credible, we conclude that convincing supportive evidence linking dry eye and infectious keratitis is minimal. For some situations, such as concomitant systemic autoimmune disease, the evidence appears a little stronger, but for the typical dry eye patient, contact lens-wearer or not, dry eye does not appear to be associated with overt microbial keratitis, even though there may be some changes in ocular flora. Further, commonly used dry eye treatments do not appear to contribute to modulating infection risk.

One problem with the existing literature is that none of the clinical studies were designed to specifically investigate a link between dry eye and infectious keratitis; rather, a link, when found, has been more an incidental finding. Epidemiological studies to specifically determine the risk of infectious keratitis in dry eye would help to provide conclusive findings. However, until such trials are forthcoming, available data indicate that dry eye (whether defined via symptom survey or a battery of clinical tests) in and of itself does not appear to be associated with an increased risk of microbial keratitis.

How then is an ocular surface that is compromised by dry eye able to protect itself? We believe that innate immune defenses are of the utmost importance in this respect. While evidence indicates that some innate defenses are breached (disrupted epithelium, reduction of some antimicrobial molecules), others appear unchanged or enhanced, including sPLA2 and AMP production. Thus, the redundancy in the innate immune system appears essential for protecting the ocular surface of dry eyes. While a number of innate mechanisms/molecules have been compared among the dry eye and the normal ocular surface, there are others (both innate and adaptive) to be investigated. Such investigations will shed further light on to how a compromised ocular surface resists infection.

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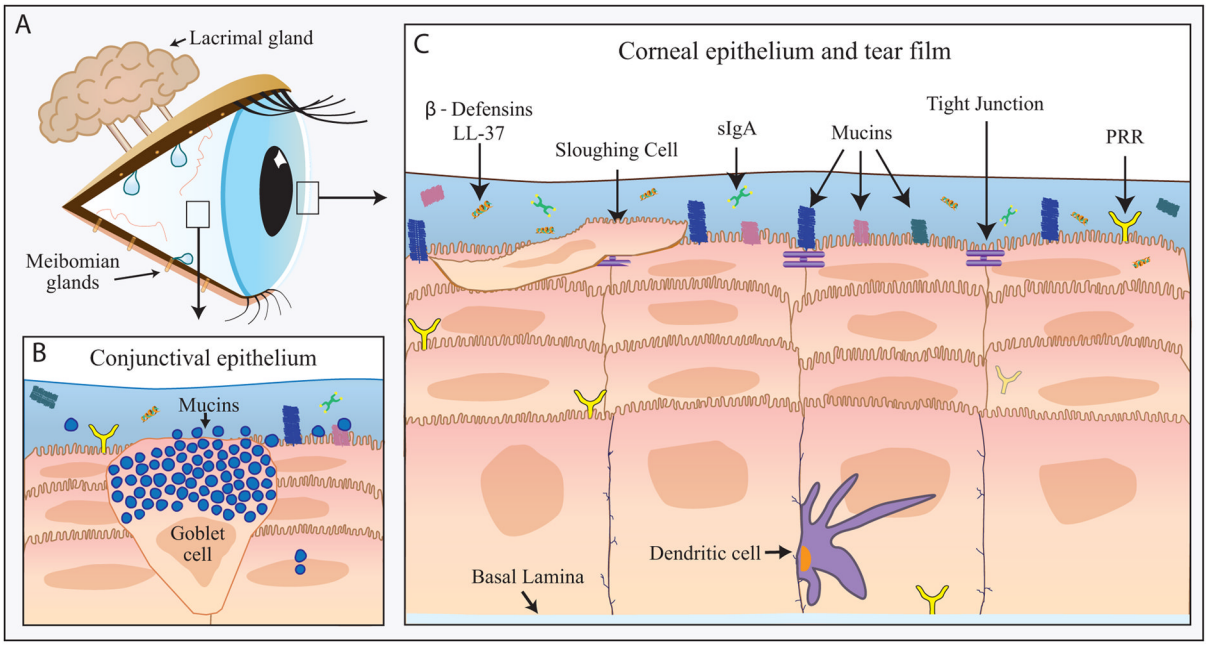

Figure 1.

Innate immune system of the ocular surface.

The innate immune system is the first line of defense against invasion for the ocular surface (A) and includes components of the conjunctival (B) and corneal epithelium and tear film (C). PRR=pattern recognition receptors, LL-37= cathelicidin, sIgA=secretory Immunoglobulin A.

Table 1

Modulation of Innate Immune Molecules in Dry Eye

Molecule	Classification of Dry Eye	Fluid/Tissue Tested	Change (References)
Lysozyme	Non-SS and SS	Tears	Decreased[114, 45–149]
	Non-SS and SS		Unchanged[150,151]
Lactoferrin	Non-SS and SS	Tears	Decreased [114,145, 146,151]
Lipocalin	Non-SS and SS	Tears	Decreased [150,151]
sIgA	Non-SS and SS	Tears	Decreased[145]
			Mixed results++[114]
	SS		Unchanged[155]
sPLA2	Non-SS and SS	Tears	Increased[163,164]
	Non-SS*	Conjunctival epithelium*	Increased[165*]
Mucins			
MUC1	Non-SS and SS	Tears, CIC	Increased[184]
MUC5AC	SS	CIC, conjunctival biopsy, tears	Decreased[167,185,179]
MUC16	Non-SS	CIC	Altered distribution/glycosylation[187]
MUC19	SS	CIC, conjunctival biopsy	Decreased[185]
AMPs			
hBD-1	Non-SS	CIC	Unchanged[198]
hBD-2	Non-SS and SS	CIC	Increased [137,198,208]
hBD-3	Non-SS	CIC	Unchanged[198]
hBD-9	Unspecified	Corneal and conjunctival impression cytology	Decreased[210]
LL-37	Non-SS	CIC	Unchanged[137]
TLRs			
TLR2	Non-SS	CIC	Increased (mRNA but not protein[136])
TLR4	Non-SS*	Corneal epithelium and stroma*	Increased[137,138]
TLR9	Non-SS	CIC	Decreased[137]

All data pertain to human studies, except where * denotes a murine model of dry eye. Tears were collected using Whatman filter paper,[114] microcapillary tube,[147,163] or micropipette,[151] Schirmer strip,[145,148,149] or surgical sponge extraction,[155] or by a tear wash.[150,179,184]

SS = Sjogren's syndrome. CIC = conjunctival impression cytology. MUC= Mucin. AMPs=Antimicrobial peptides.; TLRs=Toll-like receptors. ++ IgA was decreased in 3 of 12 eyes, and was in the low normal range for the others.