

REVIEW ARTICLE



Mucosal immunology of the ocular surface

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The eye is a sensory organ exposed to the environment and protected by a mucosal tissue barrier. While it shares a number of features with other mucosal tissues, the ocular mucosal system, composed of the conjunctiva, Meibomian glands, and lacrimal glands, is specialized to address the unique needs of (a) lubrication and (b) host defense of the ocular surface. Not surprisingly, most challenges, physical and immunological, to the homeostasis of the eye fall into those two categories. Dry eye, a dysfunction of the lacrimal glands and/or Meibomian glands, which can both cause, or arise from, sensory defects, including those caused by corneal herpes virus infection, serve as examples of these perturbations and will be discussed ahead. To preserve vision, dense neuronal and immune networks sense various stimuli and orchestrate responses, which must be tightly controlled to provide protection, while simultaneously minimizing collateral damage. All this happens against the backdrop of, and can be modified by, the microorganisms that colonize the ocular mucosa long term, or that are simply transient passengers introduced from the environment. This review will attempt to synthesize the existing knowledge and develop trends in the study of the unique mucosal and immune elements of the ocular surface.

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THE OCULAR MUCOSAL IMMUNE SYSTEM

The eye is a specialized organ with a sensory function to transmit impulses that later are transduced into vision in the brain. While specialized neurons are located at the back of the eye in the retina, the front of the eye, composed of cornea, conjunctiva, and its adnexae, including Meibomian glands, together with the lens and anterior chamber, is responsible for 2/3 of the refractive power of the eye¹. Therefore, a transparent and smooth optical surface is critical for optimum vision.

The tear film, which covers the eye, is only a few microns thick, but it maintains a moist and smooth ocular surface. It is a complex fluid composed of three different layers: aqueous, mucous, and lipid, that are constantly being produced and replenished. The tear film prevents drying of the ocular surface and functions as a lubricant between the conjunctiva and the eyelids. Also, the tear film harbors many growth factors, antimicrobial peptides, secretory IgA and vitamins, that are critical for ocular surface health by promoting immune homeostasis and host defense (Fig. 1). The conjunctival goblet cells produce mucins to moisturize and protect the ocular surface, as well as immunomodulatory factors, such as TGF- β and retinoic acid, which have been implicated in dampening immune activation of conjunctival antigen-presenting cells (APCs)^{2,3}. Muc5ac is considered the prototypic mucin on the ocular surface and goblet-cell secreted mucous is an important part of the tear film. Goblet cells will be discussed later since they are key cells maintaining an uninfamed ocular surface. Meibomian glands, which are holocrine glands located at the lid margin of the conjunctiva, produce the lipid portion of the tear film. When these glands are affected by physical or inflammatory stressors, gland dropout and

keratinization might lead to Meibomian gland dysfunction and contribute to ocular surface disease. Finally, the lacrimal gland produces the aqueous part of the tear film. This tubuloacinar gland continuously secretes water and soluble factors into the tear film. The lacrimal gland also secretes tears secondary to a foreign body on the ocular surface (reflex tears) or emotions (emotional tears). Rodents also have an extra gland called Harderian gland that also participates in tear secretion⁴.

After the tear film, the corneal epithelium is the first cellular line of defense against the environment and pathogens. The cornea is the avascular, transparent tissue whose homeostasis is dependent on trophic factors contained in fluids, such as the tear film produced by the lacrimal gland, and by the aqueous humor produced by the ciliary body. Anatomically, the most anterior portion of the cornea is a stratified epithelial cell layer with a strong barrier function, followed by the stroma composed of tightly packed collagen fibers, and finally, by organized single columnar endothelium. The function of this single columnar endothelium is to maintain corneal hydration and preserve corneal transparency.

The conjunctiva is spatially varied in structure. Close to the eyelid margin, the conjunctiva is composed of a non-keratinized stratified squamous epithelium, while close to the eye (bulbar conjunctiva) is a columnar epithelium. In mucosal tissues, lymphoid organization into follicles where APCs, T and B cells localize is thought to be important for local antigen presentation and induction of immune tolerance. (24) Peyer's patches in the intestine are the most well-characterized mucosal-associated lymphoid tissue (MALT). In the conjunctiva, in addition to intraepithelial lymphocytes, loosely organized immune cells are

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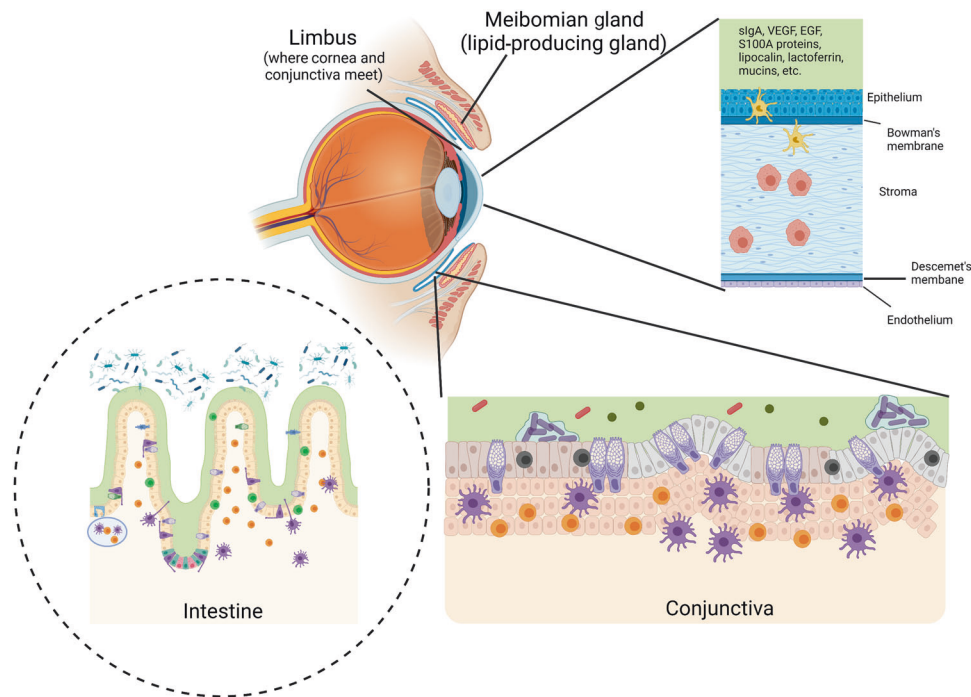


Fig. 1 Similarities and differences between intestinal and ocular surface mucosal immune tissues. Similarities between the intestinal and ocular surface mucosal immune tissues include being continually bathed in mucus, harboring a diverse array of immune cells, the presence of goblet cells, and associations with bacteria. Differences include the presence of Peyer's patches (light blue oval) in the intestines, which are not found in the conjunctiva. Also, the types, amount, and diversity of bacteria associated with the tissues is vastly different. Goblet cells (purple cells in the epithelial layer of the conjunctiva and intestines) are more concentrated in the conjunctiva as compared to the intestines. The meibomian glands associated with the eyelid supply the lipid fraction of the tears that coat the ocular surface. While both tissues are bathed in mucus, MUC5A is the tear associated mucin while MUC2 is associated with intestinal mucus. Tears contain a diverse array of factors that prevent microbes from adhering and infecting the ocular surface including: secretory IgA (slgA), VEGF, EGF, S100A proteins, lipocalin, lactoferrin, and other miscellaneous factors.

present underneath the lamina propria. More structured organized tissues forming follicles have been described as conjunctival-associate lymphoid tissue (CALT) and lacrimal drainage associated lymphoid tissues (LDALT)^{5,6}. No M cells were found in CALTs in a comparative analysis of 14 mammal species⁷. CALT and LDALT are thought to provide immune surveillance as immunoreactivity for IgA and secretoglobulins have been shown in lacrimal gland acinar cells and conjunctiva^{8,9}. CALT and LDALT are frequent findings in histology investigations in cadavers, often of advanced age. Some authors have suggested that lymphoid aggregates with B and T cells accompanying aging are pathological findings. However, a consensus on whether they are LDALTs or pathological tertiary lymphoid structures has not been achieved^{10–18}. In mice, CALTs are present in the nictating membrane and are easily missed in histological sections that include this tissue¹⁹. Intravital confocal microscopy techniques have identified CALTs in mice²⁰ that can be induced by antigen instillation^{19,21}. The number of CALTs has been shown to decrease in experimental DED but increase in allergic conjunctivitis models²².

The human and murine conjunctivae also harbor many immune cells, such as CD8⁺ T cells, innate lymphoid cells, natural killer (NK) cells, and $\gamma\delta$ T cells^{23–36}. In rodents, these intraepithelial lymphocytes are thought to participate in immune surveillance and development of conjunctival immune tolerance in homeostasis. This is borne out by functional consequences of eliminating these cells by subconjunctival injection of antibodies^{29,30}. Additionally, antibody depletion of CD8 cells during desiccation worsens dry eye phenotypes by augmenting IL-17 production by ocular surface cells and CD4⁺ T cells³⁰. The role of $\gamma\delta$ T cells in host defense of the ocular surface in relation to the commensal microbiome has been discussed. $\gamma\delta$ T cells have also

been implicated in preventing microbial keratitis, initiating corneal wound healing, and in maintaining corneal clarity^{27,28,37,38}. Some of these functions are mediated by IL-17 production^{26,38,39}. It is possible that functions performed by $\gamma\delta$ T cells in mice, may be taken on by other immune cells in the conjunctiva of humans. The functional aspects of these interactions remain to be elucidated.

APCs are widely dispersed in the lamina propria and among the epithelial cell layer in the conjunctiva. These cells express CD103, CD8, CD11b and CD11c markers and have been easily identified in human and rodent tissues^{31,40–42}. Ocular APCs have a diverse origin; macrophages, monocytes cells and plasmacytoid dendritic cells are widely present in normal conjunctiva and cornea and are important cells in dry eye, allergy, corneal transplantation and Herpes Simplex infection (which will be discussed later)^{31,41,43–47}. While expression of MHC II is an important feature of APCs, the APCs in the central cornea are MHC II negative while the cells in the peripheral cornea are MHC II⁺^{40,43}. Some of these APCs localize in close proximity to goblet cells and this will be discussed later in this review.

Innate lymphoid cell (ILCs) are an innate-like cell. ILCs can be subdivided into different subsets depending on the transcription factors and cytokines that they produce. In the gut, ILC3s have been implicated in goblet-cell hyperplasia after *Listeria* infection⁴⁸, while ILC2 cells have also been implicated in homeostatic goblet cell through secretion of IL-13³⁶. Recently, single-cell RNAseq of murine conjunctiva³⁵ demonstrated the presence of ILC2 subsets that secrete IL-13, a critical cytokine for conjunctival goblet cells. Conjunctival ILCs have also been shown to produce IL-22⁴⁹, a critical cytokine for epithelial barrier function in mucosal sites⁵⁰.

The conjunctiva is second only to the gut in density of the goblet cells that it harbors⁵¹. Although the predominant mucin produced by conjunctival goblet cells differs from the mucin

produced by intestinal goblet cells (Muc5ac vs. Muc2)⁵², conjunctival goblet cells also produce retinoic acid and TGF- β ^{2,3,53}, which together can modulate APCs that reside underneath goblet cells in the conjunctiva and can promote differentiation of T regulatory cells^{29,54,55}. Goblet cells in the gut and the conjunctiva form goblet-cell associated passages, that are critical for the sampling of antigens and establishment of mucosal tolerance^{54–57}.

Conjunctival goblet cells are exquisitely sensitive to the cytokine milieu; studies have shown that, similar to the lung, the Th-2 cytokine, IL-13, participates in homeostatic control of conjunctival goblet cells, whereas even minute quantities of IFN- γ can cause goblet cell metaplasia or cell death, and interrupt mucin secretion^{3,51,58–63}. Treatment of bone marrow-derived cells with conjunctival goblet cell supernatant alters the differentiation of dendritic cells and myeloid cells, decreases secretion of IL-12, blunts the inflammatory response to LPS and decreases APC-dependent priming of OVA-specific Th1 cells. In fact, goblet cell-conditioned medium was as efficacious as exogenous retinoic acid, a classical immunomodulatory factor also elaborated by gut APCs². Mice devoid of goblet cells due to genetic deletion of the goblet-cell associated transcription factor SPDEF, display intense conjunctival lymphocytic and myeloid infiltration and develop spontaneous dry eye disease^{55,64}. SPDEF^{-/-} mice have a higher frequency of IL-12-producing cells. In vivo administration of goblet cell-conditioned media decreases IL-12 mean fluorescence intensity in conjunctival CD11b⁺CD11c⁺ cells⁵⁵. Critical cells in conjunctival mucosal tolerance are CD11c⁺APC⁺⁶⁵ and conjunctival goblet cells^{54,55}. For more details about conjunctival tolerance, see the review by Galletti and de Paiva⁵⁷.

Together, the cornea, conjunctiva, lacrimal gland, meibomian gland, and its nerves are referred to as "lacrimal functional unit"⁶⁶, as alterations in any of these components can affect the homeostasis and the correct functioning of the others. Examples of these are dry eye disease (DED) and infection by Herpes Simplex Virus (HSV), both of which will be discussed later in this review.

UNIQUE AND SHARED MUCOSAL DEFENSES

One unique defense of the ocular surface is the constant blinking and removal of any debris. This function is shared by eyelids and tear film. While peristaltic movements advance the intestinal contents along the relatively protected lumen of the gastrointestinal tract, the eye is exposed to the environment and subject to insults such as foreign bodies. A normal blink rate is around 15–20 times per minute, but this frequency can be increased if needed (for example, a foreign body in the eye). Interestingly, a decreased blink rate is found in humans who work at video displays or computers for a prolonged time. This has been associated with an increased prevalence of DED in relatively young subjects^{67–69}.

Unlike the gut, which is literally packed with microorganisms, the conjunctival ocular surface microbiome is highly paucibacterial^{38,70–74}, but the skin of the eyelids, adjacent to the eye, is rich in microbes^{75,76}. There has been an augmented interest in microbes and their modulation of the physiology of mucosal surfaces, including the eye. The relationship between the microbiome and its effects on the eye will be discussed in the next section.

As a sensory organ, the eye is exposed to the environment and is subject to potentially harmful and inflammatory stimuli that can adversely affect vision. Because vision constitutes a strong evolutionary pressure, the eye has evolved mechanisms to limit inflammation in order to protect vision. An antigen injected into the anterior chamber in the eye is treated differently by the body than antigen injected into other organs or tissues, which illustrates a complex physiological phenomenon known as "ocular

immune privilege"⁷⁷. Immune privilege of the eye involves active as well as passive immune mechanisms that limit inflammation and minimize collateral damage to the eye. Multiple molecules and systems within the ocular fluids and on the surface of ocular cells maintain immune privilege, including, among others, TGF- β , retinoic acid, vasoactive intestinal peptide (VIP), substance P (SP), and the Fas-Fas Ligand pathway^{77–82}. Fas Ligand interacts with Fas, and leads to cell death by activating the caspase pathway⁸³. The Fas-Fas ligand signaling is well studied in the immune system, where it controls the proliferation of autoreactive T cells in the thymus (establishing central tolerance) or in the tissues (promoting peripheral tolerance)^{84,85}. Fas ligand expression on T cells after T cell receptor engagement is upregulated by TNF- α and IL-2^{86–90}. However, signaling via Fas-Fas ligand is not restricted to immune cells. Constitutive expression of Fas ligand on corneal epithelium, corneal endothelium, iris, and retina are important for maintaining immune privilege in the eye^{82,91}. Mice deficient in the Fas or FasL signaling pathway (MRL^{lpr} or gld/gld, respectively) develop lupus and Sjögren syndrome (SS)-like disease, and accumulate T cells in lacrimal glands, eyes, and conjunctiva^{92–94}.

However, unlike the inside of the eye, the ocular surface is not immune privileged and has to decide when to mount defenses or ignore (i.e., tolerate) insults. Mechanisms of tolerance on the ocular surface are of paramount importance, as uncontrolled ocular inflammation can lead to blindness. For example, corneal scarring and fibrosis after corneal insult can result in an opaque cornea that prevents light transmission, often requiring surgery to improve vision clarity. Inflammation inside the eye, also known as uveitis, can lead to retinal detachment, degeneration, and intra-vitreous hemorrhages. Studies have shown that up to 70% of patients with uveitis have permanent visual damage and meet legal criteria for blindness⁹⁵. Mucosal tolerance mechanisms have been described in all mucosal tissues, including the gut, airways and the eye⁵⁷. Conjunctival mucosal tolerance is similar to oral tolerance; an antigen administered topically into the eye prior to immunization will lead to an active immune response that generates CD4⁺Foxp3⁺ regulatory T cells or T cell anergy^{96–98}.

The ocular mucosal immune system shares some defenses with other mucosal tissues, while also displaying unique properties (Fig. 1). Examples of similar defenses to other mucosal sites are the presence of an efficient epithelial barrier composed of tight junction proteins and desmosomes, that are present in the lateral membrane of the corneal cells, maintaining a tight contact between cells. In the gut, disruption of the epithelial barrier can lead to diarrhea, bacterial translocation, and eventually sepsis. In the cornea, disruption of the corneal epithelial barrier is a hallmark of DED, and it is used clinically as both a diagnostic criterion and severity endpoint in clinical trials. In allergic conjunctivitis and DED, the production of matrix metalloproteinases such as matrix-metalloproteinase (MMP)-9 and cytokines such as IL-33 have been implicated in corneal barrier disruption by disturbing tight junction protein networks^{99,100}. Recently, barrier disruption in epithelial cells has been implicated in allergic, autoimmune, and inflammatory diseases¹⁰¹.

Thus, the ocular immune system has evolved mechanisms to maintain an optimal reflecting surface for vision while also minimizing inflammation and scarring.

MICROBIOME AND DISEASE

A host's microbiome includes bacteria, fungi, and viruses; however, this section of the review will focus on the bacterial members of the microbiome, which are the most well-characterized. It has become evident that the microbiome can dictate the status between health and disease. While this concept has been primarily investigated within the context of the intestine, there is clear evidence at other tissues where dysbiosis of the microbiome is associated with disease and pathology.

To fully understand the nature of the microbiome across various mucosal sites, one must consider the environment where bacteria may live. For example, the lumen of the small intestine (S.I.) is largely devoid of oxygen¹⁰², so the microbial components there consist largely of anaerobic bacteria and facultative anaerobes^{103–105}. Similarly to the intestinal environment, the oral cavity harbors a diverse microbiome in an environment with large amounts of mucus. At this site, the microbiome is made up of over 700 species of bacteria of which 99% come from the phyla, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, and *Spirochaetes*^{106–108}. Conversely, other sites in the host offer different environments, which allow for varying consortia of bacteria to thrive. For example, the skin is much drier and has fewer nutrients than the intestine and is continually exposed to a drier environment and has a low abundance of nutrients compared to other mucosal surfaces. That being said, the composition of the skin microbiome is largely dependent on how oily or moist an area is^{109–111}. While the intestine and skin have higher densities of bacteria, areas like the lungs also appear to harbor a resident microbiome, albeit in much lower quantities. The lungs are still exposed to the environment; however, the resources for bacteria are much less abundant due to a relative lack of nutrients. While many agree that commensal bacteria are present within the lungs, a consensus on what constitutes a healthy microbiome in that location still lacks resolution^{112–115}.

The ocular mucosal environment brings together characteristics shared with each of the aforementioned areas. The eye is bathed in mucus like the intestines and oral cavity; however, nutrients are quite limited similar to the skin and lung habitats. Moreover, the eye microbiome is considered low biomass like the lungs, and it has been calculated that there is about 1 bacterium for every 20 ocular epithelial cells¹¹⁶. Notably, the eye is a relatively hostile environment for potential colonizing microbes due to the continual flow of tears that wash the ocular surface, and the elevated levels of salt, antimicrobial peptides, antibodies, and other antimicrobial factors like lysozyme. Further, the action of blinking helps to prevent bacteria and other pathogens from adhering to the corneal surface. These mechanisms are relatively effective at preventing colonization of the central cornea, which is normally devoid of bacteria, even under desiccating conditions that lead to dry eye^{117,118}. However, the environment appears more hospitable to bacteria underneath the eyelids. Here, the impact of moving eyelids is felt much less and there is less disruption of colonization by physical force. Underneath the eyelid on conjunctival epithelia is where the first identifications of eye-colonizing microbes were found^{38,118}.

Forming a consensus on defining the “core” ocular microbiome has been difficult, largely due to the paucibacterial nature of the eye¹¹⁶. Studies dating back to the 70s using traditional culture techniques have shown that up to 50% of conjunctival swabs do not yield culturable bacteria⁷⁵. However, the development of next generation sequencing has provided an opportunity to sample larger cohorts of patients and establish appropriate pipelines of analyses to eliminate contaminating genomic reads that could dramatically disrupt the true identities of ocular microbes. *Corynebacterium*, *Propionibacterium*, and coagulase-negative *Staphylococci* are all routinely sampled from the conjunctiva^{76,118–122}. Intriguingly, torque teno virus is a human virus linked to cancers and other diseases that is found in a high frequency of sampled individuals¹¹⁶. This constituent of the virome may influence ocular health and disease; however, the development of models centered around torque teno virus remains elusive.

Ocular microbiome studies have compared the constituents of the ocular microbiome between healthy individuals and contact lens wearers, or patients with pathologies like keratitis, DED, conjunctivitis, and ocular surface allergy^{70,119,121,122}. Information acquired from these studies shows that the composition of the microbiome changes with the health status of the eye; however,

true mechanistic and causative studies have yet to be performed. Our recent study showed the immune effects of the local microbiome at the ocular surface. Specifically, the eye-colonizing *Corynebacterium mastitidis* (*C. mast*), induced the recruitment of IL-17-producing $\gamma\delta$ T cells to the conjunctiva where they play a direct role in enhancing the ability of the ocular surface to resist infection by pathogens³⁸. While *C. mastitidis* and other *Corynebacterium spp.* have been detected on the ocular surface of humans^{119,123–125}, the role that these microbes play in local ocular surface immunity in humans has not been investigated.

The local microbiome is not the only consortium of microbes that affect the eye. The intestinal microbiome is also closely linked to ocular immunity. Germ-free mice, which are devoid of all microbes, do not generate a sufficient level of secretory IgA (sIgA) antibodies^{11,126}, which bind and facilitate the washing away of potential pathogens and allergens. Similarly, a series of studies, which combined observations from humans and mouse models, investigated the critical role that intestinal microbes play in the development and severity of DED, illustrating the far-reaching effects of intestinal dysbiosis^{70,127,128}. Intriguingly, a correlative study showed this may not only apply to DED, as intestinal dysbiosis was also linked to development and severity of keratitis^{129,130}.

The intestinal microbiome not only affects the ocular surface, where it is difficult to exclude direct transfer of gut bacteria onto the eye as a result of poor hygiene, it also can modulate intraocular disease. Microbes within the intestine can activate autoreactive T cells that target the retina and trigger autoimmune uveitis in a genetically predisposed model^{105,131}. In addition, microbes can provide antigen-independent stimuli through their immunoregulatory metabolites in an immunization-induced model¹³². In humans, dysregulated microbiome signatures correlate to disease in glaucoma and age-related macular degeneration^{133–137}. While immunological mechanisms for intraocular diseases have been defined in animal models of uveitis, mechanisms of disease in models such as glaucoma and age-related macular degeneration are still much less well understood.

Understanding the influence that the microbiome has on ocular disease, opens up the potential for modulating the microbiome to limit pathogenesis. In models of Sjögren Syndrome (SS), fecal transplants were successful at reducing disease severity^{127,128}. Similarly, antibiotic treatment that eliminates bacteria which may, directly or indirectly, activate autoreactive uveitogenic T cells, ameliorated experimental uveitis^{131,132}.

Bacteria that can colonize the eye could be genetically modified to produce and secrete factors that can modulate local host immunity to encourage ocular health. Because these bacteria would be continuously delivering the therapeutic substance to the eye, the need for frequent and laborious application of eye drops could become unnecessary. Of course, more details like biological “switches” within the bacteria would need to be defined so that there would be optimal control over drug delivery. Overall, these studies and hypotheses reveal a potential for using the microbiome to revolutionize the way eye diseases are treated.

NEUROIMMUNE AXIS AND THE MODULATION OF MUCOSAL IMMUNITY

Despite its small size, the cornea is the most densely innervated tissue in the body^{138,139} (Fig. 2). Uniquely, it is primarily innervated by sensory nerves¹⁴⁰. Evidence shows that sympathetic and parasympathetic nerves reach the ocular surface; however, these “non-sensing” nerves do not pass the limbus—where the mucosal conjunctival tissue meets the optically clear cornea—and do not enter the cornea during homeostasis¹⁴¹. In other, more well-studied, mucosal tissues like the intestines, the nature of the interplay between the host immunity and neurons is becoming more apparent. Moreover, this relationship

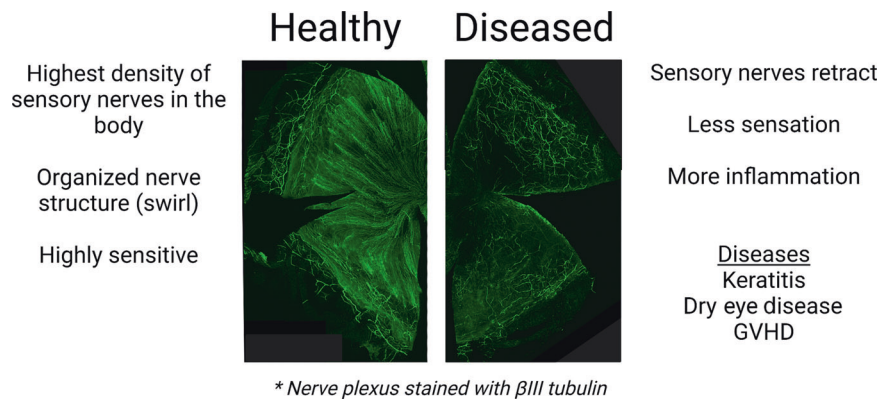


Fig. 2 Ocular surface diseases can lead to a loss of sensory nerves in the cornea. In a normal corneal, thick sensory nerve trunk begin at the limbus (peripheral to the cornea) and innervate the cornea eventually thinning to become fine sensory nerve endings which terminate in the corneal epithelium. Fine sensory nerve endings become more concentrated and begin to swirl as they reach the central cornea (left). During ocular surface disease, sensory nerve endings can retract (right) and this results in a loss of blink reflex that can be temporary or permanent. Loss of blink reflex can leave the eye susceptible to infection, dry eye phenotypes and excessive inflammation. Nerves in the figure were stained with β III tubulin.

contributes in a major way to the balance between tissue homeostasis and pathology.

As already mentioned, unlike the intestines, the cornea is an extremely sensitive tissue. The reason for that is that the eye is continually exposed to the external environment, and sensation is a means to prevent injury. During steady state, corneal nerves express the transient receptor potential (TRP) channels, TRPV1, TRPA1, TRPM1, and TRPM8, which can respond to heat (TRPV1), pain (TRPV1), cold (TRPA1/TRPM1/TRPM8), and chemical stimuli (TRPM1/TRPM8)^{142–146}. Notably, TRPV4 is also expressed at the cornea; however, it is more closely associated with the apical domain of corneal epithelial cells, where TRPV4 is essential in forming tight junctions^{146,147}. In addition to TRP channels, the ocular surface also expresses acid sensing ion channels (ASIC), which detect changes in pH due to noxious stimuli and/or damage associated with inflammation or trauma^{144,148}. Generally, upon activation of these channels, the eye responds by blinking and/or tearing which allows the movement of tears across the eye. This response to stimuli is meant to dislodge and wash away potential pathogens, allergens, or irritants, which drain into the tear duct and are removed from the eye. The process of blinking is critical for the maintenance of mucosal homeostasis at the ocular surface, and it is documented that the loss of blink reflex results in ocular surface drying, increased susceptibility to infection, and potentially blinding pathology¹⁴⁹. Corneal nerves do not only mediate the physical act of blinking, they also appear to contribute to the appropriate production of tears. Recently, lacrimal gland organoids from mice and humans were created from lacrimal gland biopsies and revealed that the release of the aqueous portion of the tears was mediated by neurotransmitters like norepinephrine, VIP and pilocarpine¹⁵⁰. Similarly, in humans, the loss of corneal nerves correlates with atrophy of the meibomian gland, which, as mentioned above, supplies the lipid fraction of the tears¹⁵¹.

Neuropeptides contribute to maintaining ocular immune homeostasis and, when dysregulated, can exacerbate disease. For example, sensory nerves in the cornea express substance P (SP) and SP promotes vascularization and inflammation^{152,153}. Calcitonin gene-related peptide (CGRP) is another factor that influences corneal homeostasis by increasing wound healing and promoting tissue healing phenotypes in macrophages (M2)^{154,155}. Conversely, CGRP is also known to play a role in photophobia and may limit the ability of the cornea to resist infection^{156,157}. Vasoactive intestinal peptide (VIP) is another neuropeptide produced at the ocular surface, and this factor has been shown to aid in limiting disease in diabetic keratopathy, bacterial keratitis,

and allergic conjunctivitis^{158–161}. Other neuropeptides like neuropeptide Y (NPY) are present at the ocular surface¹⁶²; however, their roles in ocular surface disease and homeostasis are less clear.

A tissue with similar mechanisms at play is the lung, which is another mucosal tissue directly exposed to environmental stimuli. TRPV1 neurons have been extensively studied in this tissue and have been shown to greatly modulate the immune response. Activation of TRPV1 channels in lung neurons by noxious elements, allergens, or other factors can lead to lung irritation and coughing¹⁶³. Specifically, activation of TRPV1 neurons during lung infection dampens immunity to *Staph aureus*, leading to morbidity in mice. Ablation of this neuronal subset increased survival¹⁶⁴. Conversely, TRPV1 expression is increased in models of extended lung pathology^{165–167} leading to the conclusion that TRPV1 neurons can be both anti-inflammatory and pro-inflammatory, depending on the context. At the ocular surface, TRPV1 expression is upregulated after corneal injury¹⁶⁸. Furthermore, activation of the nociceptive receptor, TRPV1, resulted in increased susceptibility to *Pseudomonas aeruginosa* infection¹⁵⁷; however, TRPV1 signaling also appears to appear to prevent the adhesion of environmental bacteria¹⁶⁹.

The skin also harbors neurons with nociceptors; however, skin is not exclusively innervated with sensory nerves like the eye. Instead, the skin is innervated by a pool of sensory, sympathetic, and parasympathetic nerves. In this tissue, TRPV1-expressing neurons have also been demonstrated to modulate immunity¹⁷⁰. Specifically, stimulation of these neurons, through sensing of bacterial or fungal infections, leads to the development of “anticipatory” immunity, which enhances anti-microbial immunity at sites that are peripheral to the site of infection. This mechanism relies on successful transfer of neuronal signals from the periphery to the neuronal cell bodies, and back down other branches of the ganglia¹⁷¹. Another recently described mechanism of neuronal control of skin immunity focuses on sensory nonpeptidergic neurons, which express the Mas-related G-protein-coupled receptor D (MrgprD). These nerves suppress mast cell responses and limit excessive mast cell-centric immunity¹⁷². Under pathological conditions like dermatitis, the direct sensing of cytokines and pathogens can lead to itch and disease^{173,174}. Notably, MrgprD and MrgprA3 nerves exist within the conjunctiva, suggesting that these nerves play an active role maintaining ocular homeostasis through sensing “itch” signals leading to blinking and tearing¹⁷⁵. The roles that these and similar receptors play in ocular homeostasis and disease have yet to be fully investigated.

The enteric nervous system governs the control of smooth muscle throughout the intestinal tract. The enteric nervous system

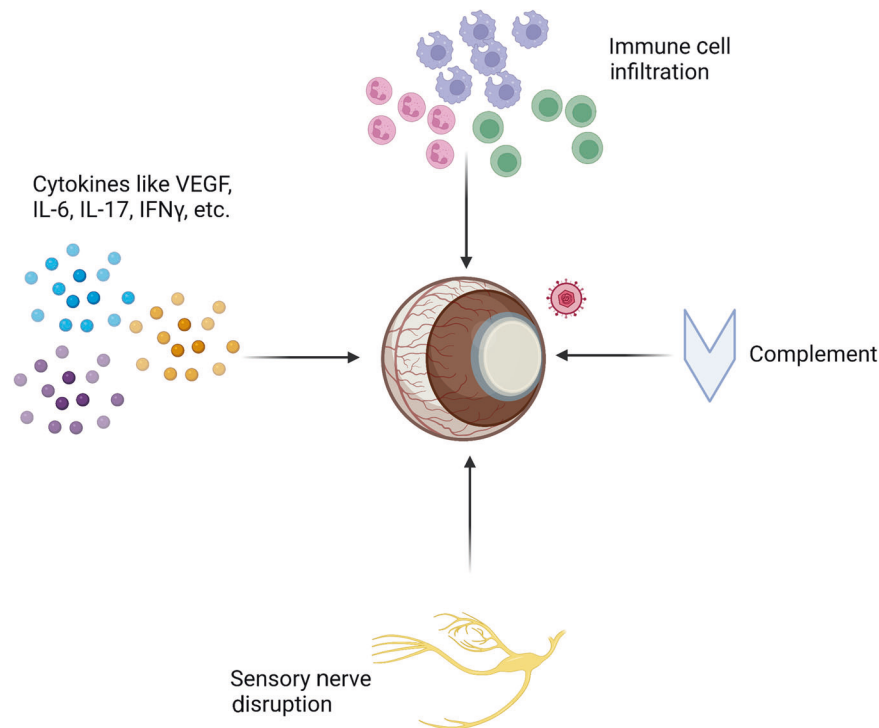


Fig. 3 Factors that contribute to Herpes Stromal Keratitis (HSK). After a predominantly sub-clinical primary infection, HSV-1 gains access to sensory neurons and enters a latent state within trigeminal ganglion neurons indefinitely. Spontaneously or during times of immune suppression, HSV-1 can reactivate and virions can be deposited at the cornea resulting in an inflammatory response. Repeated reactivation events result in: (1) increased recruitment of inflammatory immune cells, (2) the production of inflammatory cytokines/chemokines, (3) disruption of sensory nerves, and (4) the initiation of complement, which preferentially targets sensory nerves. Because HSK is a multi-factorial disease, these processes all contribute to disease and ultimately result in tissue damage, fibrosis, and eventual blindness.

mostly consists of autonomic sympathetic and parasympathetic nerves. Due to the extensive number of immune cells within the intestinal tract and their close proximity to enteric nerves, there are ample opportunities for the neuroimmune axis to function to maintain tissue homeostasis and prevent infection and disease. The enteric nervous system secretes various neurotransmitters and neuropeptides that are known to modulate immunity such as acetylcholine (ACh), NE, VIP, and SP¹⁷⁶. Through feedback mechanisms with immune cells, these transmitters and peptides can modulate cytokine production within innate and adaptive immune cells. Some of these factors have been studied in relation to the maintenance of corneal nerves and immune response; however, not all have been thoroughly investigated. Intestinal-resident immune cells also can regulate neuronal survival after infection. Specifically, muscularis macrophages can protect neurons from death through an arginase 1-polyamine axis that depends on β 2-adrenergic receptor stimulation¹⁷⁷. In addition to the enteric nervous system, enteric glial cells assist in regulating intestinal barrier permeability¹⁷⁸. This could have implications for the eye, in that intestinal bacteria have been identified in the eye and have been posited to play a role in pathologies like age-related macular degeneration and other intraocular diseases^{135,179}; however, these claims require more substantiated support before definitive conclusions can be made.

CORNEAL HERPES INFECTION AND HERPES STROMAL KERATITIS

Common infectious agents of the eye are herpesviruses, which include varicella zoster virus (VZV) and HSV type 1 and 2. For the purposes of this part of the review, we will focus on HSV-1, which is the leading cause of blindness due to infectious disease in developed countries^{180,181}. HSV-1 and other herpesviruses can

initiate lasting pathology due to their ability to cause corneal scarring, which necessitates a corneal transplant or will lead to blindness. At the periphery, HSV-1 normally infects sub-clinically and replicates within corneal epithelial cells. This allows the virus to gain access to sensory neurons, which are supplied by the trigeminal ganglion. The virus can then enter a latent state within the trigeminal ganglion and will remain in the trigeminal ganglion for the life of the host. Under the influence of various stimuli, the virus can reactivate from latency and produce live, infectious virions, which are delivered to and induce immune responses at distal sites (cornea)^{182–188}. These immune responses, rather than viral replication, are the primary reasons for pathology and disease. While the blinding effects of corneal herpesvirus infections are clear, herpesviruses can infect and cause disease at an array of barrier and mucosal interfaces. Therefore, a thorough understanding of the immune responses directed against herpesviruses is critical for the development of better therapies to alleviate pathology in the eye as well as other sites of infection like the genital tract and skin.

The prevailing consensus is that the cornea lacks lymphatics and is largely devoid of immune cells, which reduces the chances of developing excessive inflammation that leads to immunopathology. Especially in mice, the tears are effective at washing away viruses and preventing infection, and most mouse models of ocular HSV-1 rely on scarifying the cornea to facilitate infection. Upon infection with HSV-1, corneal dendritic cells (DCs) and macrophages become activated and respond to the virus by producing type I interferons (IFN α and IFN β) and inflammatory cytokines/chemokines, IL-6 and CXCL1/9/10^{189,190}. Similarly, epithelial cells respond to HSV by producing inflammatory cytokines, which recruit other immune cells, like neutrophils and inflammatory monocytes to the cornea, which likely migrate from the conjunctiva¹⁹¹.

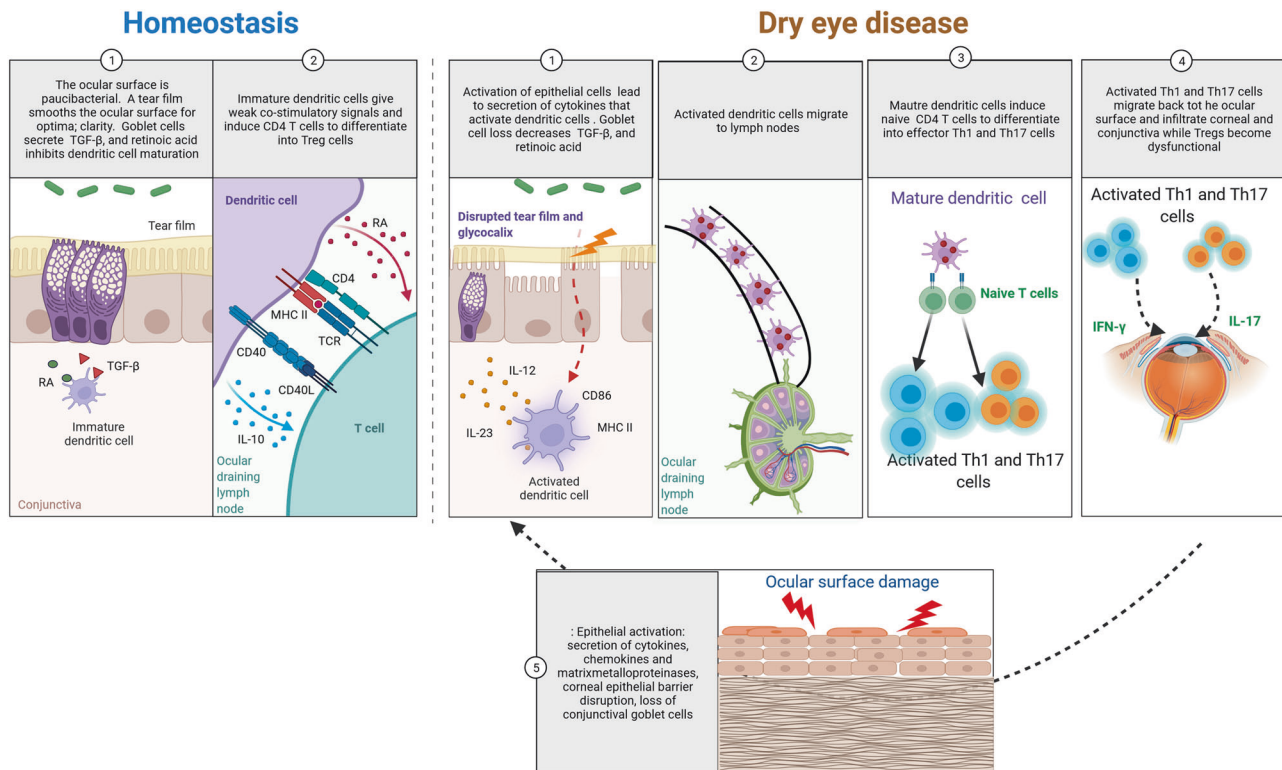


Fig. 4 The immune response in dry eye disease and the vicious cycle of inflammation. Homeostasis: 1-In homeostasis, the ocular surface is paucibacterial and has innumerable anti-microbial defenses, including a healthy tear film rich in anti-microbial proteins (SA100s, lysozyme). In the conjunctiva, goblet cells secrete retinoic acid (RA) and TGF-beta that maintain the resident dendritic cells in an immature state. 2-Immature dendritic cells provide ocular surveillance and migrate constantly to the ocular draining nodes where regulatory T cells (Tregs) are preferentially primed. dry eye disease: 1-Insults to the ocular surface cause activation of epithelial cells, disrupted tear film and glycocalyx which in turn lead to secretion of chemokines, secretion of T helper (Th) polarizing cytokines such as IL-12 and IL-23 and upregulation of CD86 and MHC II, leading to activated dendritic cells. Loss of goblet cells and squamous metaplasia of the conjunctival epithelium lead to goblet cells that do not properly secrete at the ocular surface. Secondary to loss of goblet cells, there is less RA and TGF- β , leading to further APC activation. 2-Activated dendritic cells migrate to ocular lymph nodes. 3-Mature dendritic cells prime naive T cells into Th1 and Th17 cells. 4-Activated Th1 and Th17 cells migrate back to the eye, where they secrete IFN- γ and IL-17, which cause goblet cell loss, corneal barrier disruption and activation of innate immunity. Dysfunctional Tregs also participate in the immune response of dry eye disease (not depicted). 5- Insults to the ocular surface reinitiate the vicious cycle of dry eye activating epithelial cells, disrupting the tear film and glycocalyx which in turn lead to secretion of chemokines, secretion of Th polarizing cytokines such as IL-12 and IL-23 and upregulation of CD86 and MHC II, leading to activated dendritic cells. Loss of goblet cells and squamous metaplasia of the conjunctival epithelium lead to goblet cells that do not properly secrete at the ocular surface. Secondary to loss of goblet cells, there is less RA and TGF- β , leading to further APC activation.

As the virus enters latency days after primary infection, migratory DCs begin priming the adaptive CD4⁺ and CD8⁺ T cell responses. Within days of primary infection, HSV-specific CD8⁺ T cells infiltrate the trigeminal ganglion and assist natural killer (NK) cells in pushing HSV-1 into a latent state by producing IFN- γ and lytic granules containing granzyme B¹⁹²⁻¹⁹⁵. This process results in a life-long battle between HSV-specific CD8⁺ T cells and HSV-harboring neurons. When CD8⁺ T cell functionality is compromised through immune suppression, HSV-1 can exit latency, travel back to the cornea and cause disease¹⁹⁶⁻¹⁹⁸.

Unlike CD8⁺ T cells, which largely traffic to the trigeminal ganglion from draining lymph nodes, CD4⁺ T cells expand after primary infection and traffic to the trigeminal ganglion and cornea. In the trigeminal ganglia, CD4⁺ T cells support CD8⁺ T cell functionality and may help limit viral spread¹⁹⁹. However, CD4⁺ T cells that migrate to the cornea play the opposite role^{200,201}. Specifically, in concert with inflammatory monocytes, cornea CD4⁺ T cells can produce vascular endothelial growth factor-A (VEGF-A). Additionally, the HSV-1 protein, infected cell polypeptide (ICP)4, can directly activate transcription of VEGF-A, suggesting that HSV-1 itself can induce the production and release of VEGF-A²⁰². Since VEGF-A can stimulate vascularization of the cornea, this factor plays a direct role in the infiltration of neutrophils and

inflammatory monocytes that disrupt the clear, organized collagen network within the cornea, leading to corneal scarring. Depletion of CD4⁺ T cells during this process can mitigate subsequent waves of leukocyte recruitment to the cornea, reducing pathology and disease²⁰³. In mice, pathology occurs after acute primary infection, and does not necessarily rely on viral reactivation from latency. In humans, however, this process is progressive, taking years to develop after numerous rounds of viral reactivation from latency.

Conventionally, the cause of corneal pathology was thought to be restricted to the immune response directed against reactivated virus that was deposited in the cornea. More recently, this line of thought has been challenged by observations that corneal hypoesthesia (loss of sensation) accompanied pathology, suggesting a role for the nervous system in disease. Indeed, after HSV-1 infection, sensory nerves retract; however, this is not unique to HSV-1 as DED, ocular manifestations of Graft versus Host Disease (GVHD), and corneal wounds, also result in the retraction of sensory nerves (Fig. 3)²⁰⁴⁻²⁰⁸.

In other models of disease sensory nerves can grow back, albeit sometimes in reduced numbers. However, in HSV infection, sensory nerves are replaced by "non-sensing" sympathetic nerves that perpetuate the loss of sensation indefinitely¹⁴⁰. Loss of

sensation results in an inhibited blink reflex and desiccation stress, which acts to further enhance inflammation in a feed-forward manner causing more severe disease. The well-established procedure of tarsorrhaphy (sewing the eyelid shut) can prevent the desiccation and alleviate disease.

Nerve disruption is common during infectious diseases and has been observed in models of intestinal disease where disruption of nerves can result in reduced motility and symptoms associated with “irritable bowel syndrome”. At the ocular surface, the contribution of corneal nerve disruption during HSV pathogenesis is beginning to gain interest and understanding. CD4⁺ T cells recruited to the cornea produce complement factor C3, which contributes to sensory nerve retraction and loss, and inhibition of this factor using cobra snake venom alleviates disease. This mechanism is not only functional during HSV infection, but it likely extends to other ocular surface diseases, as ocular manifestations of GVHD is known to be mediated through this pathway as well²⁰⁷. Additionally, VEGF-A, produced by monocyte-derived cells and CD4⁺ T cells, and is better known to induce blood vessel formation, was shown to act directly on corneal nerves to disrupt sensory nerves and induce the ingrowth of sympathetic nerves²⁰³. Macrophages and monocyte-derived cells have the potential to cause pathology in the cornea due to their production of inflammatory cytokines and disruption of corneal nerves; however, macrophages can act to prevent neuronal loss in the intestine¹⁷⁷, which highlights an interesting difference between these two mucosal tissues. The mechanisms that lead to the production of VEGF-A in each of these immune cell populations has yet to be elucidated; however, both monocyte-derived cells and CD4⁺ T cells closely localize to sympathetic nerves during pathogenicity, suggesting that a tripartite relationship exists to perpetuate inflammation.

Antimicrobial components of tears have an ability to target all potential pathogens. For example, beta defensins—produced by corneal epithelial cells²⁰⁹—can inactivate bacteria, fungi, and viruses^{210,211}. Similarly, lactoferrin is known to sequester iron, which can impede growth of bacteria and fungi²¹². Additionally, lactoferrin can degrade RNA and acts as an antiviral. Most recently, it has been shown to limit SARS-CoV2 infection²¹³. Lipocalin is another factor present in tears that possesses robust antimicrobial activity. Its endonuclease activity can act as an antiviral, and it can bind siderophores to prevent bacterial and fungal infection^{214,215}. Antibodies can bind and neutralize HSV-1 to limit its ability to infect the eye and persist there. Antibody responses are generated after primary HSV infection, and these antibodies do confer protection against disease. In the skin, IgM that binds HSV innately, can protect the host from skin lesions and death²¹⁶. In the eye, however, B cell deficiency leads to longer viral persistence after primary infection that can increase viral spread to areas like the brain²¹⁷. After primary infection, induced antibodies play a larger role in neutralization of infectious virus. Others have shown that antibodies generated in response to an HSV-1 vaccine can reduce the need for functional adaptive T cells in preventing viral reactivation¹⁹⁷. Also, in models of HSV-1 reactivation, neutralizing antibodies can lead to the under-reporting of viral reactivation from latency. Specifically, we have observed that only 30% of mice that are reactivating HSV-1 actively shed infectious virus, while the remaining 70% of mice have HSV-1 antigens deposited in the cornea that are detectable by fluorescence microscopy. Further, antibodies from HSV-infected dams can be passively transferred to pups through nursing^{218,219}. Those antibodies have been shown to prevent HSV-1 infection and dissemination to the brain, which can lead to cognitive disorders.

DRY EYE DISEASE

A disease that often affects the ocular surface is DED, which affects millions of people worldwide and is the most common reason to

seek eye care. DED is a chronic disease that, in extreme cases, can lead to blindness secondary to corneal melting/perforation. Patients complain of burning, itching, grittiness and ocular pain to various degrees of intensity, from mild discomfort to debilitating pain. However, it is well established that a disconnect between clinical symptoms and clinical signs happens in dry eye and some of these effects might be secondary to nerve alterations in severe cases. Female sex, increased age, presence of systemic autoimmunity, use of contact lenses are all risk factors that have been associated with dry eye. In addition, any disturbance in the lacrimal functional unit can lead to DED.

DED is a heterogeneous disease. For example, SS, an autoimmune disease that primarily affects the exocrine glands such as lacrimal and salivary glands, is the prototypic aqueous-tear-deficient DED. Patients with SS tend to have more severe forms of dry eye. Meibomian gland disease, which is also another form of DED, happens when the Meibomian glands are involved and have an unstable or a decreased lipid layer that leads to tear instability and increased evaporation. Alterations in eyelids and incomplete closing at night can also lead to dry eye. Another contributing factor is reduced blinking while staring at computer displays and smartphone devices for extended periods of time and use of systemic medications that have secondary drying effects.

Despite its low frequency, evidence has shown that commensal bacteria can have a protective role on the ocular surface³⁸. Studies have indicated that topical antibiotics administered as eye drops into the conjunctival sac can modulate the ocular microbiome differently from systemic antibiotics^{220,221}. The body of evidence identifying the protective role of the microbiome during dry eye comes from experiments in mice using either germ-free animals or evaluating their ocular surface after oral antibiotic-induced dysbiosis (see recent reviews^{222,223}). Naive germ-free mice have low levels of IL-1 β , lower levels of sIgA, extremely decreased number of conjunctival goblet cells, and increased frequency of APCs and IL-12-producing dendritic cells^{11,70,126,128,220,224}. This is accompanied by corneal barrier disruption that is more prevalent in the C57BL/6 germ-free female mice, and can be reverted by fecal microbiota transplant from conventional, specific pathogen-free mice¹²⁸. Administration of oral antibiotics worsens the dry eye phenotype in mice subjected to desiccation stress and also increases the production of inflammatory transcripts such as IL-1 β , IL-12, TNF- α in corneas subjected to topical LPS challenge^{70,224}. Further evidence of the protective effect of the microbiome was observed in the CD25^{-/-} SS model, where germ-free CD25^{-/-} mice had more accelerated eye and worse lacrimal inflammation than CD25^{-/-} mice with a conventional microbiome. IL-12, MHC II and IFN- γ were identified as key players in the lacrimal gland in this model¹²⁷. IL-12 antibody depletion in germ-free CD25^{-/-} mice ameliorated the lacrimal gland inflammation, confirming the role of Th1 cells in this model¹²⁷. Interestingly, and in contrast to most other autoimmune and inflammatory disease models, where depletion of microbiota protects from disease, germ free or MyD88^{-/-} non-obese diabetic (NOD) mice develop worse Type I diabetes than their counterparts with a conventional microbiome^{225,226}. Conversely, administration of antibiotics to NOD mice, which also develop salivary gland inflammation, ameliorates this inflammation^{227,228}, indicating that context dictates the role that the microbiome plays in disease.

It is also well established that dry eye is accompanied by a vicious cycle of inflammation, activation of innate and adaptive immune responses and damage to the epithelium, which further causes inflammation (Fig. 4). The exact source of the initiating event(s) in dry eye have not been identified, although it is clear that there are multiple entry points (autoimmunity, aging, low humidity exposure, video display, use of contact lenses, dysbiosis) that can combine to constitute “multiple hits.” An insult on the ocular surface (desiccation, surgery) can lead to

epithelial activation, which increased production of inflammatory cytokines and MMPs, activation of inflammasome and oxidative stress pathways, altering the local environment. Clinical signs are altered corneal barrier function and loss of conjunctival goblet cells. Activation of APCs, migration to eye-draining lymph nodes, priming of Th1 and Th17 cells that migrate to the ocular surface epithelium and secretion of IFN- γ and IL-17, causing further insult to the ocular surface, breaking the corneal barrier, and activating the epithelium, reinitiating the vicious circle and becoming self-perpetuating (Fig. 4)^{229,230}. Interruption of APC migration, achieved by subconjunctival administration of liposome-encapsulated clodronate (which depletes APCs), or lymphadenectomy of eye-draining nodes will decrease the generation of eye-pathogenic T cells^{231,232}. The subject has recently been extensively reviewed—the interested reader is referred to reference²³³ for a comprehensive review of dry eye pathophysiology.

Unifying events in the vicious circle of dry eye are disturbances in the tear film (quantity, quality, mechanical spread), hyperosmolarity and epithelial activation. As such, a hyperosmolar tear initiates a cascade of pro-inflammatory cytokine secretion by the cornea and conjunctival epithelium^{230,234–236} and activation of inflammasome and oxidative pathways. Defects in autophagy, endoplasmic reticulum stress and the unfolded protein response have all been implicated in dry eye, and are supported by the increased expression of the chaperone GPR78 in animal models and in culture systems^{62,237}. Increased expression of the chaperone GPR78 in epithelial cells is also noted in minor salivary gland biopsies in SS patients and in impression cytology conjunctival imprints^{62,238}.

Epithelial cells that line either the conjunctiva and cornea, or acinar and epithelial ducts from SS and dry eye patients, are active players in dry eye. They suffer bystander damage by immune cells that become activated and secrete cytokines such as IFN- γ and IL-17^{58,62,239–244}. Corneal and conjunctival epithelial cells express low levels of MHC II, but can upregulate it after stimulation with IFN- γ ^{60,239,245}. A similar phenomenon²⁴⁶ is seen in the lacrimal and salivary glands and also by intestinal epithelial cells²⁴⁷. It has been proposed that these epithelial cells can function as non-professional APCs^{245,247,248} although a consensus regarding their signaling pathways and the type of CD4⁺ T cells that are modulated by these MHC II⁺ cells, has not been reached²⁴⁷. SS and non-SS patients have increased levels of HLA-DR in their conjunctiva^{239,249–252} and this has been used as a biomarker in clinical trials^{249,253}. Experimentally, constitutive expression of major inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) pathway due to genetic deletion of its transcriptional regulator I κ B- ζ , induces an SS-like disease with both ocular and glandular involvement²⁵⁴. Further evidence of epithelial cells being initiators of dry eye is provided by experimental dry eye models where epithelial disturbance through either desiccation or topical application of benzalkonium chloride elicits an innate response, which is followed by an adaptive immune response^{30,58,97,255}. Other inflammatory pathways that have been implicated in dry eye are MAPK (JNK, ERK and p38)^{236,256,257}. For example, studies have shown that JNK2^{-/-} mice are resistant to dry eye-induced corneal changes²⁵⁸. Activation of innate pathways such as Toll-Like receptors, NOD, MyD88, and NLRP3 in corneal epithelial cells has also been implicated in dry eye^{259–263}, further supporting the crucial role of epithelial activation in DED.

There is a complex relationship between APCs and conjunctival goblet cells²⁶⁴. Goblet cells and their products not only act to mechanically lubricate the eye, but also actively contribute to immune homeostasis at the ocular surface by secreting TGF- β and retinoic acid, as mentioned before^{29,30,58,62,231–233,240,243,265,266}. Studies have shown that experimental dry eye changes the polarization of conjunctival macrophages from M2 to M1^{267,268}.

Desiccating stress was also associated with disappearance of immature monocytes in the conjunctiva and their replacement with MHC II⁺ macrophages, suggesting active recruitment of these cells to the conjunctiva²⁶⁸. This was also accompanied by elevated expression of genes involved in antigen presentation and inflammation like NLRP3²⁶⁸. Furthermore, at least five of the differentially expressed genes (*Icam1*, *Batf*, *S100a9*, *Mx1*, and *Irf7*) evaluated by a NanoString® panel from murine conjunctivae subjected to desiccating stress were also upregulated in the conjunctiva of SS patients²⁶⁹. Some of these genes have been implicated in the SS and dry eye before^{270–279}. Of particular interest is BATF. Although not causally implicated in SS or dry eye, BATF is a transcription factor involved in differentiation of Th17 cells²⁸⁰ whose signature cytokine, IL-17, promotes corneal barrier disruption^{240,243}.

It is therefore not surprising that the only four FDA-approved drugs to treat dry eye are immunomodulatory agents that interfere with the vicious cycle of dry eye by modulating T cell activation and by decreasing immune synapse (cyclosporine [in two different formulations], lifitegrast and loteprednol etabonate)^{281–284}. While much has been learned about dry eye disease in the last 20 years, effective therapies that can be used by a large number of patients remain elusive.

CONCLUSIONS

In summary, the ocular surface is a complex mucosal system that is highly specialized to perform the functions of maintaining ocular physical and immunological integrity. The component cells, immune as well as structural, interact with each other and with the environment, including physical, chemical and microbial stimuli. Any defect in one of the cellular or secretory components in this complex interacting network can trigger, or can contribute to, ocular surface pathology. Research during the past decade, employing state-of-the-art approaches, has revealed much of these critical networks and interactions and has opened new therapeutic perspectives, but much remains to be learned. The microbial and neuroimmune aspects controlling ocular surface health and disease have been foci of intense interest and promise to yield novel clinical approaches to treatment of ocular surface disease.

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AUTHOR CONTRIBUTIONS

R.R.C. conceptualized and edited the manuscript, C.S.d.P. and A.J.St.L. wrote the manuscript, all authors refined and approved the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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