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Visions of Eye Commensals: The Known and the Unknown About How the Microbiome Affects Eye Disease

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Abstract

Until recently, the ocular surface is thought by many to be sterile and devoid of living microbes. It is now becoming clear that this may not be the case. Recent and sophisticated PCR analyses have shown that microbial DNA-based “signatures” are present within various ethnic, geographic, and contact lens wearing communities. Furthermore, using a mouse model of ocular surface disease, we have shown that the microbe, *Corynebacterium mastitidis* (*C. mast*), can stably colonize the ocular mucosa and that a causal relationship exists between ocular *C. mast* colonization and beneficial local immunity. While this constitutes proof-of-concept that a bona fide ocular microbiome that tunes immunity can exist at the ocular surface, there remain numerous unanswered questions to be addressed before microbiome-modulating therapies may be successfully developed. Here, the authors will briefly outline what is currently known about the local ocular microbiome as well as microbiomes associated with other sites, and how those sites may play a role in ocular surface immunity. Understanding how commensal microbes affect the ocular surface immune homeostasis has the potential revolutionize how we think about treating ocular surface disease.

Keywords

immunity; ocular disease; ocular microbiome

1. Introduction

The body is home to trillions of microorganisms at virtually all barrier surfaces and the effects of these microorganisms can extend beyond the surface to internal organs like the kidney, heart, and brain. This consortium of microbes can include fungi, viruses, and protists; however, the most extensively studied group to date, is bacteria. While the gastrointestinal tract contains the largest number and greatest diversity of bacteria, other

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Conflict of Interest

The authors declare no conflict of interest.

sites like the skin, vagina, oral cavity, and the nasal cavity are also known to harbor a consistent level of bacteria.^[1]

Recently, causal relationships of the intestinal microbiota on biological phenomena like immune tolerance, obesity and metabolic syndrome, inflammatory bowel disease (IBD), inflammatory arthritis, and various autoimmune diseases, have been elegantly illustrated in the laboratory setting by using germ free and gnotobiotic facilities, antibiotics, and fecal transplants.^[2–6] Because the effects of manipulating the intestinal microbiome are so far-reaching, it has been difficult to attribute immunological roles to local commensals such as those found at the ocular surface. Here, we will review the microbial communities found at the ocular surface, and discuss the effects that gut and ocular bacteria may have on ocular surface immunity and disease.

2. Extraintestinal Manifestations of Inflammatory Bowel Disease (IBD): Linking the Gut to the Eye

IBD is a chronic inflammatory disease that encompasses Crohn's disease and ulcerative colitis, which affects the small/large intestines and the colon, respectively. Like many inflammatory disorders, the cause(s) of IBD are not entirely known; however, there is strong evidence to suggest that host genetics, luminal content composition including the microbiome, and environmental triggers all play a role. In up to 40% of IBD cases, inflammation may extend beyond the gut to peripheral sites like the skin, joints, and eyes. Approximately 10% of extraintestinal IBD cases are accompanied by manifestations in the eye including episcleritis, uveitis, and conjunctivitis.^[7,8]

Many host genes associated with IBD are also involved with sensing bacteria. Therefore, the gut microbiome, which consists of bacteria, viruses, fungi, and protozoans, is believed to play a major role in IBD pathogenesis. Thus far, bacteria have garnered the most attention as they make up the most substantial part of the gut microbiome.

Studies using germ-free mice, which lack all bacteria, have shown that under normal circumstances commensal bacteria mature and tune the developing immune system as well as subsequent immune homeostasis. Mice lacking commensal bacteria fail to develop mature lymphoid tissues, are deficient in the production and secretion of the secretory antibody IgA and antimicrobial peptides, and cannot properly control cytokine production.^[9–11] In addition, sensing of the microbiota by epithelial cells ensures barrier integrity and proper regulation of mucus production, which enhances the physical separation between microbes and the host, reducing the likelihood of bacterial translocation and infection. Finally, commensals tune the local T cell response in the gut, which was shown with the discovery that segmented filamentous bacteria (SFB) colonize the intestine of mice and profoundly affect the immune landscape within the intestine. Specifically, when these bacteria colonized the lumen of the intestine, interleukin (IL)-17 producing cells were activated and recruited to the intestine, and modulated host defense mechanisms.^[12] Elegant studies in mice showed a causal relationship between this specific microbe and resistance to the enteric pathogen *Citrobacter rodentium*. Conversely, however, later studies linked the presence of SFB and possibly other bacteria to exacerbated forms of autoimmunity.^[13,14]

The reach of the commensal microbiome extends beyond the local environment to distal sites. For example, antibiotic treated or germ-free mice display major defects in the generation and activation of innate immune cells in the bone marrow and beyond, leaving peripheral tissues particularly susceptible to infection by other pathogens like fungi. At the ocular surface, germ free mice are susceptible to *Pseudomonas aeruginosa* infection due to lack of a well-developed ocular surface immunity.^[15] However, when the animal is monocolonized with coagulase negative staphylococcus sp., resistance to ocular infection is restored, suggesting that the commensal microbiome enhances the establishment of ocular surface host defense. It must be noted that a common issue in monocolonization studies is that the microbe of interest begins to occupy niches it does not normally occupy making definitive conclusions about localized immunity difficult.^[16] Therefore, with the above study, the authors could not conclude that either the intestinal or ocular bacteria were contributing to protection from *P aeruginosa*. Alternatively, in a model of spontaneous uveitis, germ free and antibiotic treated mice develop disease less frequently and less severely compared to normally housed controls kept in specific pathogen free (SPF) conditions.^[17] Also, further illustrating the influence of the gut microbiome on ocular disease, dysbiosis of gut flora has recently been correlated with Sjögren's Syndrome (SS) in mice and humans.^[18]

3. Commensal Bacteria and Intraocular Disease

Commensals may have a role in modulating immunity and disease not only on the ocular surface, but also in the neuroretina. Recently, our group demonstrated a role for the intestinal microbiome in triggering autoimmune retinal disease, in a mouse model of spontaneous autoimmune uveitis, an intraocular inflammation that leads to retinal destruction and blindness. The R161H strain of mice was engineered to express a transgenic T cell receptor specific to retina and serves as a model for human autoimmune uveitis. We showed that the retina-specific T cells, which should remain quiescent during steady state conditions due to lack of contact with their antigen that resides behind the blood retinal barrier, are stimulated within the intestine by an apparent antigenic mimic from intestinal bacteria.^[17] A similar finding was reported in mouse models of multiple sclerosis and autoimmune diabetes.^[19,20] Furthermore, a number of human studies reported mimicry between gut bacteria and candidate antigens involved in human autoimmune diseases.^[21,22] In line with the notion that specific antigens are being recognized by the immune system, several major histocompatibility complex molecules have been linked to various systemic and ocular autoimmune diseases.^[23] Specifically, in uveitis, human leukocyte antigen (HLA) complexes have been implicated in disease to include birdshot chorioretinopathy, associated with HLA-A29,^[24] Vogt-Koyanagi-Harada disease, associated with HLA-DQw3,^[25] and acute anterior uveitis, associated with HLA-B27. Interestingly, HLA-B27 has also been linked with other systemic autoimmune diseases and was shown to affect the makeup of the intestinal microbiome.^[26] In contrast to this evidence for antigen-dependent effects, other studies suggested antigen-independent effects of gut microbiome, due to metabolite changes and/or Treg induction. Nakamura et al.^[27,28] reported that mice actively immunized for uveitis and treated with antibiotics had expanded regulatory T cells in parallel to reduced uveitis severity. Although a direct causal relationship was not established, these findings

suggest that changes in gut microbes led to induction of (microbiota specific?) Tregs, which in turn affected disease progression independently of the autoantigen. In the aggregate, these findings support the notion that the commensal microbiome plays a role in the development and outcome of a variety of autoimmune diseases.

It has been suggested that the microbiome may play a role in other retinal diseases, such as age-related macular degeneration (AMD) and glaucoma, where immune mechanisms have been implicated. Although mice lack a macula, they do develop some delayed age-related features of AMD (AMDf), and researchers have used mouse models to predict that inflammation, smoking, and diet promote disease. Recently, a group linked a high glycemic diet to an increase in AMDf compared to mice fed a low glycemic diet.^[29] Computational analyses showed a complete alteration of the intestinal flora between high and low glycemic diets and strongly correlated microbial metabolites as a protector against AMDf. In the next step, it will be important to perform functional studies on these metabolites to definitively show how microbial components can modulate disease.

Glaucoma is an intraocular disease that leads to progressive optic nerve degeneration, loss of visual acuity and eventual blindness. Under normal conditions, aqueous humor is continually produced and drained within the eye, but under glaucomatous conditions, aqueous humor is not properly drained, resulting in elevated intraocular pressure (IOP). Even though the disease is primarily attributed to increased IOP, the correlation is far from absolute. Individuals with elevated IOP may not develop disease, and some patients with normal IOP may still develop disease. It is believed that once disease begins, local inflammatory responses can exacerbate glaucoma; however, systemic infections do not appear to contribute to disease outcome.^[30] While overproduction and inadequate drainage of aqueous are well-known causes of high IOP, certain medications and eye trauma may also be contributors. Interestingly, a group has linked the composition of the oral microbiome as a factor in glaucoma.^[31] Specifically, increased bacterial loads in the oral cavity and increased TLR4 activity correlated with disease of the retina and optic nerve. Knowing that the oral and ocular microbiomes contain similar bacteria, it is tempting to hypothesize that the ocular microbiome may also be linked to disease. Several mouse models of glaucoma are available and may open the way to future studies on the connection between commensal bacteria and glaucomatous disease.

Research investigating the role of the microbiome in AMD and glaucoma are still in nascent stages, so more studies are needed before definitive links can be made. Correlative human studies and causative animal studies solidifying the links between disease and microbes will certainly push this field forward to, potentially, reveal novel microbiome-based therapies that alleviate disease.

4. Influence of Microbiome on Ocular Allergy?

The well-known hypothesis that the microbiome influences susceptibility to allergy has been well-supported by epidemiological and microbiological studies. An array of studies has linked allergic disease in children to Caesarian birth, formula feeding, and antibiotic consumption during pregnancy.^[32,33] These conditions affect the intestinal microbiome

during a crucially formative time in the immune system development. Administration of probiotics can establish or re-establish immune homeostasis within the gut and, subsequently, the periphery, reducing allergy-associated phenotypes. Also, early feeding of known allergens, like peanuts, to children, has been shown to reduce the chance of food allergy later in life, which suggests that age and the state of the microbiome matter greatly during exposure to potential allergens.^[34] Species of *Clostridium*, *Bacteroides*, and *Bifidobacterium* are linked to regulatory responses in the intestine and correlate with less severe allergy.^[35–37] In the eye, it is still uncertain if and how the ocular surface microbiome or intestinal microbiome influences allergic conjunctivitis and other allergic ocular diseases. Recently, a group showed that conjunctival CD11b⁺ dendritic cells (DCs), which recognize and process ocular commensals, can mediate fibrosis in an ocular model of allergy, suggesting that commensal sampling by CD11b⁺ DCs may play a role in ocular allergy.^[38]

Furthermore, it is conceivable that other bacteria live in similar niches to that of *C. mast.* and modulate immunity in vastly different ways. We and others have found that $\alpha\beta$ T cells, natural killer cells, antigen presenting cells (APCs), basophils, eosinophils, and others exist at the ocular surface,^[39] and it is possible that commonly found strains of ocular bacteria like Coagulase Negative Staph (CNS), *Propionibacterium Acnes*, and others may stimulate different types of immune cells including those found in allergic disease models. It is certainly worth investigating how *C. mast* and other strains of bacteria may affect type 1 (antiviral) or type 2 (allergic) immunity throughout life. We have circumstantial evidence that ocular mucosal immunity develops quite early in life, similar to that found in the gut. Therefore, it is likely that the nascent microbiome directly or indirectly influences ocular surface diseases that develop later in life.

5. The Defense Mechanisms at the Ocular Surface

The ocular surface is continually exposed to environmental factors such as microbes, potential allergens, and toxins. Similar to other mucosal sites, the eye has developed an array of mechanisms to effectively interact with the environment and limit infection as well as autoimmunity. Other than the blinking eyelid, the first, and probably most effective, protector of the ocular surface is the tear film. Within the tear film are antimicrobials, which can kill pathogens on contact while also stimulating other innate immune cells to begin warding off pathogens. Antimicrobials are short peptide sequences (<100 AA), which are electrostatically charged and induce bacterial death by binding and forming pores in bacterial cells walls. However, anti-microbial peptides have also been shown to stimulate the functionality of innate immune cells, illustrating the versatility and importance of these peptides in preventing disease at the ocular surface. Other components of the tear film include secretory Immunoglobulin A (sIgA), which binds and facilitates the removal of allergens and/or pathogens, and mucins/lipids that lubricate the ocular surface and reduce the chance for abrasions caused by microtraumas as well as impede adherence of microorganisms.^[40] In addition, the constant production and drainage of tears efficiently washes away most factors that may cause ocular disease.

The next line of defense comes in the form of the conjunctiva, a thin mucosal tissue that lines the eyelids and extends onto the eye to the peripheral cornea. Studies from animals and

post mortem eye samples have revealed that the conjunctiva has the characteristic elements of a mucosal barrier site and harbors a diverse repertoire of immune cells that include M cells and APCs, which can process antigen, antibody producing B cells, and innate and adaptive T cells, which can produce cytokines and/or directly kill pathogens.^[41–43] Also, corneal epithelial cells act as stores for IL-1 α , which can be passively released after trauma and/or damage to trigger immune responses that would limit bacterial invasion.^[44] These cells, can also produce the potent inflammatory cytokine, IL-8, after stimulation with neuropeptides secreted by corneal nerves, which can sense damage to the ocular surface.^[45] Corneal stromal cells produce endogenous anti-microbial proteins to prevent the invasion of pathogens.^[46–48] Ocular keratocytes, after stimulation with proinflammatory cytokines, can produce IL-6 and defensins to limit spread of bacteria after infection. These mediators also contribute to the recruitment of neutrophils, which can effectively limit infectious disease at the ocular surface, but can also, when not appropriately regulated, cause immunopathological damage to the cornea. Finally, embedded within the cornea are DCs and macrophages that continually sample the local environment, so appropriate defense or healing mechanisms are initiated in response to an infectious or a traumatic event.^[49,50] When functioning correctly, these combined factors effectively protect the ocular surface from most insults and the eye remains in a state of homeostasis.

6. The Ocular Microbiome-Myth or Fact?

The existence of a “true” resident ocular microbiome has been debated by ophthalmologists, with no clear consensus being reached. During steady state, the central cornea is sterile and swabs routinely yield no viable bacteria. In fact, even in cases of infection-caused corneal ulcers, cultures routinely yield nothing.^[51] This, however, only suggests that the cornea is a hostile environment for potential microbes probably due to the mechanisms outlined in Section 5. There are other areas of the eye, like the conjunctiva, that appear more permissive to microbes and routinely harbor quantifiable, albeit low, numbers of live bacteria.^[52]

Analyzing the ocular surface (conjunctival) microbiome remained a difficult task for several reasons. First, like the lungs, the conjunctiva is a low biomass tissue, meaning that the total numbers of quantifiable bacteria are several orders of magnitude less than those found in higher biomass areas like the intestine and even the skin. In addition, simple swabbing of the conjunctiva and plating on agar plates made it difficult to distinguish if the bacteria were resident to the eyelids, the conjunctiva, or happened to land on the ocular surface from the air, or were deposited from skin by rubbing the eye. While conventional culture methods yielded a “core” microbiome consisting of various *Staphylococci* spp., *Propionibacterium*, and diphtheroids, more sophisticated deep sequencing analyses have revealed a more complicated situation. Early reports using 16S rRNA analysis suggested a very limited diversity of bacteria at the ocular surface with only 12 genera of bacteria being maintained across four healthy controls.^[53] However, more recent studies have shown that greater than 500 genera of bacteria can be identified from conjunctival swabs. From these studies, it appears that factors like geography, ethnicity and contact lens wear, all affect microbial communities at the ocular surface. That being said, clear trends are being revealed in that “commensal” bacteria like *Corynebacterium* and coagulase negative *Staphylococci* as well as “pathogenic” bacteria like *Pseudomonas* are found in relatively high abundance among

samples from several studies. Depending on the study, these genera make up about 30–70% of bacteria at the ocular surface.^[54–59] More support for the existence of a true ocular microbiome comes from two studies that show a significant difference in the microbial signature at the ocular surface compared to surrounding facial skin, suggesting a clear distinction between the two sites. Interestingly, contact lens wear appears to normalize the microbial communities between the eye and the surrounding tissues.^[54,56] It will be interesting to identify bacterial factors unique to these microbes that allow them to remain at the ocular surface or “outcompete” other microbes across various populations. Similarly, identifying other factors, environmental or host-derived, that determine the composition of less abundant ocular microbes may be beneficial to our understanding of transient bacteria at the ocular surface. While, in the aggregate, these data were suggestive of an ocular surface microbiome, DNA-based surveys cannot distinguish live bacteria from dead bacteria. Therefore, formal proof of a living, resident microbiome was still lacking.

Such formal proof was provided by our isolation of what is apparently a bona fide commensal, *Corynebacterium mastitidis* (*C. mast*), from the conjunctival tissue of C57BL/6 mice that were housed in our animal facility at NIH, or in the facility of a collaborator from another Institution, but not in mice purchased from commercial vendors, which was instrumental in showing the consequences of the presence of this bacterium on local host defense. It should be noted that it is difficult to prove that a bacterium found on the ocular surface is a long-term resident that lives there, as opposed to a transient guest transferred from skin or feces that is on its way to elimination by the antibacterial conditions of the ocular surface. This is especially true in view of the very low bacterial biomass detectable on the ocular surface. Fortuitously, this organism was not only absent in mice from commercial vendors, but also could not be acquired by them through casual contact with “our” mice, even after extended co-housing. *C. mast* could only be acquired through purposeful inoculation, or perinatally from the dam, whereupon it persisted on the ocular surface apparently indefinitely, indicating true commensalism rather than self-reinoculation. The same difficulty in distinguishing a resident commensal from a transient guest is still holding back the conclusion whether the lung possesses a true microbiome. That said, functional consequences on local immunity and host defense, which are a hallmark of mutually beneficial commensalism, can probably be achieved, at least in part, by contact with transient bacteria that do not persist as true commensals.^[60]

C. mast appears to be uniquely able colonize the ocular surface compared to other bacteria, including some other strains of *Corynebacterium* (Figure 1). First, it persists for many weeks after inoculation. Second, it elicits production of IL-17 from conjunctival $\gamma\delta$ T cells, which elicits downstream mechanisms that protect the ocular surface from infection by other, potentially pathogenic, bacteria and fungi (and likely keeps the commensal itself in check).^[60] Interestingly, after colonization, *C. mast*, a rod-shaped bacterium, begins to form filaments, suggesting that: 1) it is physically attached to conjunctiva and 2) it is under physiological stress. However, it is unclear whether *C. mast* forms a biofilm on the conjunctiva. From these data, we can hypothesize that the immune response elicited by *C. mast*, which prevents pathogens from taking hold, also controls the commensal itself. In future investigations, it will be important to understand what allows *C. mast* to resist the antimicrobial nature of the ocular surface. A possible explanation is that the lipophilic nature

of *Corynebacterium spp.*^[61] and the high lipid content of tears^[62] creates an environment suitable for *C. mast* growth. Once this and other aspects of the *C. mast*/ocular surface environment are known, perhaps genetic manipulation of *C. mast* may allow for the long-term and selective expression of therapeutic proteins or molecules at the ocular surface, which has already proven efficacious in studies involving the intestines.

Dysbiosis of commensal bacteria is routinely correlated to various diseases. The nature and impact of dysbiosis in relation to the ocular surface is only beginning to be studied. As noted above, conserved commensal “signatures” at the ocular surface have emerged between various geographic, ethnic, and contact lens wearing groups; however, the causal relationships and implications of these findings are neither straightforward nor fully understood. For example, the diversity and number of bacteria increases at the conjunctiva in a spontaneous mouse model of SS in thrombospondin-1 (TSP-1) knockout mice as they age and develop disease, due to an inability to efficiently phagocytose microbes.^[63] While the causative mechanism(s) for disease are not fully understood, one can hypothesize that bacteria are directly causing the pathology. Alternatively, due to an inability to clear bacteria, an aberrant immune response to select commensal(s) may lead to tissue destruction, which may then open more niches for bacterial growth.

We have demonstrated the importance of IL-17-regulated genes like S100A8/9 at the ocular surface; however, the importance of sIgA should not be underestimated. At the ocular surface and other mucosal tissues, sIgA remains a critical mediator of host defense by binding viruses, toxins, allergens, bacteria, and other potential irritants. Secretory IgA, which is produced by B cells in lymphoid tissues and at the periphery, is dynamically regulated by the microbiome. Mice treated with antibiotics or raised in a germ-free environment fail to generate an effective repertoire of sIgA and are susceptible to infections at barrier surfaces. At the ocular surface, it appears that the intestinal and ocular microbiome cooperatively control the tear sIgA, which is required for optimal protection of the ocular surface from infection.^[64]

Despite bacteria being the most well-characterized aspect of the microbiome, the term “ocular microbiome” would also include any colonizing fungi or viruses. Similar to bacteria, analysis of these microbes yielded varied results across studies. For example, one study revealed that fungi and viruses make-up less than 2% of the microbial reads from conjunctival swabs.^[57] In another study, DNA analysis showed that 65% of healthy subjects were positive for torque teno virus, which has also been linked to culture-negative cases of endophthalmitis.^[54,65] Other viruses have also been found at the ocular surface of asymptomatic subjects, suggesting that niches for viruses may exist at the ocular surface.^[66] Now that these less prominent components of the microbiome are recognized, it is critical to begin teasing out if these viruses are modulating disease, if immune responses against these players are eliciting immunopathology, or if the viruses are acting as an adjuvant to boost the immune response against other stimulants.

7. Can Ocular Commensals Become Pathobionts?

Our studies have identified *C. mast* as a beneficial commensal which tunes the immune response at the ocular surface and protects from pathogenic infections. However, *Corynebacterium spp.* have the potential to cause serious eye infections in the elderly and the immune suppressed. This highlights the thin line between a beneficial commensal and a pathobiont. In the elderly, immunosenescence contributes to the development of cancer, reduced resistance to infection, and inability to control commensal bacteria.

Immunosenescence can take one of many forms, including reduced inflammatory cytokine production, monocyte differentiation, and neutrophil functionality,^[67] all of which are important for the prevention of ocular infection.^[60] This, therefore, suggests that *Corynebacterium spp.*, and possibly other bacteria that act as commensals in the steady state, may become pathobionts under times of moderate to severe immune deficiency. In a recent study investigating the conjunctival microbiome of humans with or without trachoma caused by *Chlamydia trachomatis*, the authors suggest that non-chlamydial bacteria may be responsible for disease.^[68] Specifically, they link *Corynebacterium spp.* to this disease because *Corynebacterium* and *Streptococcus* were both found in higher abundance among diseased patients. Development of appropriate animal models should help to elucidate the active mechanisms behind the phenomena described above.

Dry eye disease (DED) affects around 10% of adults in the United States, has reached over 30% in Asian countries, and causes serious issues in the elderly.^[69] While a definitive etiology is yet to be delineated, it appears that two main mechanisms are: 1) decreased lacrimal and/or Meibomian gland function with age, which results in reduced quantity and quality of tears and/or chronic inflammation at the ocular surface triggered by poorly defined mechanisms. Common treatments include the frequent use of artificial tears to lubricate and anti-inflammatories to control the ocular surface inflammation. In an attempt to boost the anti-inflammatory nature of the host's immune system, multiple groups have aimed to enhance T regulatory (T_{reg}) cells, which can suppress excessive immune responses and potentially limit disease.^[70,71] Indeed, while boosting the T_{reg} response is beneficial in some models, the question of what is stimulating the inflammatory response in the first place, has not been sufficiently assessed.^[72] We propose that inadequate control of commensal flora at the ocular surface, especially in the elderly, may lead to dysregulated inflammatory responses. It is also an intriguing question whether Meibomian gland dysfunction (MGD), which is a major cause of DED in humans, is affected by ocular surface microorganisms. On the one hand, like other glandular tissue, Meibomian glands and lacrimal glands are highly innervated and rely on neural outputs for proper functionality. On the other hand, nerves and immune cells routinely interact and modulate each other. An ocular example of this comes from a study linking systemic muscarinic acetylcholine receptor (mAChR) blockade with DED and T_{reg} dysfunction.^[73] In future studies, it will be intriguing to identify, using animal models, if the neuro-immune/microbiome axis can influence not only the inflammation at the ocular surface, but also lacrimal and Meibomian gland functionality in DED and other disease models.

Notably, the ocular mucosa appears to be routinely exposed to pathogenic bacteria. PCR-based studies have revealed that the ocular microbiome consists of a mix of known

commensals and pathogenic bacteria to include *P. aeruginosa*, *Staphylococci* (including the methicillin-resistant strains).^[53,55,56,59] As with many infections, other than traumatic stress, it is difficult to ascertain how and when a pathogen in an apparent state of quiescence will become actively infectious. Identifying how commensals may modulate cellular mechanisms governing these processes could help bolster host defense at the ocular surface.

Paradoxically, not only a suppressed immune system but also an overactive immune system can allow a commensal to behave as a pathobiont, resulting in a similar phenotype. While under conditions of immune homeostasis an IL-17 response is beneficial and protects from pathogens, in models of DED and various forms of keratitis, IL-17 is highly pathogenic, and its neutralization by antibodies or suppression by corticosteroids or mTOR inhibitors leads to the alleviation of the characteristic symptoms of disease.^[74–76] Data from animal models have suggested that in these diseases adaptive CD4⁺ T cells are primed against a self or unknown antigen in the draining lymph node to produce IL-17, migrate to the eye, and cause tissue destruction.^[77] However, it is also possible that $\gamma\delta$ T cells or other immune cells that are reactive to tissue-resident commensals may contribute pathogenic IL-17 due to aberrant priming of commensal-dependent immunity under conditions of traumatic/desiccating stress, or some other unknown mechanism.

Another set of diseases that manifest at the ocular surface are autoinflammatory syndromes, which result from genetic mutations leading to the overproduction of innate factors primarily due to an overactive inflammasome. Manifestations include red eyes, visual impairment, and severe ocular discomfort. These diseases are referred to as periodic fever syndromes or cryopyrin-associated periodic syndromes (CAPS), and include neonatal onset multisystem inflammatory disease (NOMID), familial cold autoinflammatory syndrome (FACS), Muckle-Wells syndrome (MWS). While anti-inflammatory treatment strategies have proven beneficial, the triggers of inflammation remain unidentified. Mouse models bearing homologous mutations to those found in humans have revealed involvement of innate pattern recognition receptors (PRRs) that lie upstream of the inflammasome complex and provide a “priming” signal to the complex. PRRs include toll-like receptors (TLRs), nucleotide binding oligomerization domain (NOD)-like receptors (NLRs), and others, all of which can all be activated by microbial products (as well as host “alarm” signals and some synthetic compounds). Here, again, mouse models have proven essential in our understanding of disease, as mice treated with systemic antibiotics have a reduced incidence of inflammasome-mediated disease in the skin and other areas, implicating the commensal flora at these sites.^[78,79] It is still unclear; however, if this same relationship applies to the ocular surface. With the more intense study of the ocular microbiome and candidate ocular commensals, it should not be long before we know what activates the inflammasome in the ocular mucosa and causes ocular pathology associated with autoinflammatory disorders.

8. Conclusion

The nature of the eye and its continual exposure to the surroundings creates a unique environment for state-of-the-art analysis of ocular function to include the shape, size, and clarity of the cornea, the ability of the retina and optic nerve to capture and process signals from light, and the interpretation of those signals by the brain. In a similar vein, our work,

together with the work of several other labs, has opened up a new area of research that will have clear implications for ocular surface and intraocular health. Sophisticated gene analyses revealing commensal “signatures” have provided invaluable correlative data, which will form the framework of future studies. Primed with these data, we can now begin to perform well-controlled causative studies, so that future therapeutics may soon be within reach. Specifically, the focus should be centered upon the development of probiotic or probiotic-like therapies, which would be able to modify the immune landscape at the ocular surface to optimize resisting infection and autoimmunity. The recent use of microvesicles for delivery of therapeutics in animal models has proven efficacious in animal models of glaucoma. It is not unreasonable to think that bacterial products can be loaded into these microvesicles to deliver a low-level of immune stimulation, which would boost ocular surface immunity. Further, it will be worthwhile to study the colonization properties of individual strains of bacteria. As mentioned earlier, *Corynebacterium spp.* appear beneficial under normal circumstances but can become pathogenic under immune suppressed conditions. Once we have an idea of the roles of individual strains of bacteria, we can then begin to modulate the representation of those communities to reduce the chances of a commensal becoming a pathobiont while also maintaining ocular surface immunity. In conclusion, we have only just begun investigating the impact of the microbiome on ocular disease, and there is still much to be learned. We are embarking on a journey that will revolutionize how we treat ocular disease and will have a definite potential to create truly innovative therapies to preserve ocular health.

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Corynebacterium (mastitidis and others)
Staphylococci
Propionibacterium
Torque Teno Virus

Prevalent on Conjunctiva:

Pseudomonas
Acinetobacter
Methylobacterium

Figure 1.

The conjunctiva and eyelids are colonized with differing consortia of microbes. A representative image of where major components of the ocular microbiome may reside. [54,56,60]