Vision is one of the most important, core senses; what we see allows us to connect with and understand our surroundings. As the population continues to age and vision declines, the need to further our understanding of ocular diseases remains imminent. One of the recent and most exciting advancements in the field has been the investigation into how microbial organisms and their metabolic products influence the eye.

Communities of various microorganisms, known as microbiomes, exist throughout the human body, from our skin to our gut to many other organs. The ocular surface microbiome (OSM), found on the cornea, conjunctiva, eyelids, and lacrimal and meibomian glands, and the gut microbiome, housed within the gastrointestinal tract, are the two key players in the context of ocular disease.

The OSM protects the ocular surface through a variety of mechanisms. For example, it acts as a physical barrier between external microbes and the eye while chemically protecting the eye by producing antimicrobial molecules for tear film.

Although the gut microbiome is not directly anatomically related to the eye, it too plays a large role in ocular health by regulating homeostasis and immune function. A healthy metabolism and a robust gut barrier prevent the translocation of pathogenic microbes and metabolites from the gut into systemic circulation, and resultant downstream inflammatory damage in other organs such as the eye.

For both the OSM and gut microbiome, maintaining a diverse microbiome is essential for creating a balance of organisms with proinflammatory and anti-inflammatory properties. This balance can be affected by various external elements, including aging, diet, lifestyle, medications, and environmental factors. When this balance is skewed, also referred to as dysbiosis, inflammatory and pathogenic processes dominate. The reviews highlighted in the Microbiome and Ocular Health thematic issue of The American Journal of Pathology cover a large range of ocular diseases, provide meaningful insights into the microbiome—ocular relationship, and describe associated changes in microbial composition, microbial metabolites, and the effects of microbiome manipulation to advance our understanding of the role of the microbiome in ocular diseases. The highlighted diseases include glaucoma, age-related macular degeneration, uveitis, inherited retinal degenerations, retinal vascular diseases, and retinopathy of prematurity (ROP).

**Glaucoma**

Chen et al describe the impact of the gut microbiota on the development of glaucoma, a disease marked by the progressive degeneration of retinal ganglion cells (RGCs) and their axons, leading to irreversible vision loss. The glaucomatous state is associated with a specific microbial profile: a rat model of glaucoma has reported an increased *Firmicutes/Bacteroidetes* ratio at the phyla level, which is correlated with an increase in taurochoic acid metabolites (negatively affecting RGC function) and a decrease in...
Microbiome in Ocular Diseases

Age-Related Macular Degeneration

Xiao et al17 explored microbiome composition, metabolic profiles, and dietary effects in age-related macular degeneration (AMD). It has now become apparent that there is no singular microbiome signature that points to the risk of AMD progression. In a case-control study comparing patients with AMD versus control patients, the AMD patients exhibited increased Prevotella (associated with mucosal inflammation and T helper 17 immune response), Holdemanella, and Desulfovibrio (associated with stimulation of the gut immune response).8 A separate study showed that patients with neovascular AMD have microbiota enriched in Anaerotruncus (linked to proinflammatory signaling), Oscillibacter (associated with dysregulation of tight junction components, affecting gut permeability), Ruminococcus torques, Eubacterium ventriosum, and an increased Firmicutes/Bacteroides ratio.9 The relative increase of Firmicutes is heavily driven by an expansion in Clostridia, which has been associated with a greater risk for AMD. These microbial shifts in patients with AMD are associated with enrichment of genes involved in purine degradation and arginine biosynthesis, which influence AMD pathogenesis.

Xiao et al10 also describe several studies that compare germ-free (GF) mice (with complete absence of microbiota) versus specific pathogen—free mice (with conventional microbiome) and measure transcriptomic and phenotypic changes. GF mice exhibited altered retinal and retinal pigment epithelium/choroidal transcriptomes, with many differentially expressed genes, including Vegf, Pparc1a, Angpt1, and Tie1, players in inflammation and angiogenesis. In a laser-induced model of choroidal neovascularization (a hallmark of neovascular AMD), GF mice displayed reduced lesion size and decreased peripheral microglial activation around the lesion compared with specific pathogen—free mice.

This review also explores several core studies that examine the effects of a high-fat diet and high glycemic diet on microbiome composition and AMD development. One key study showed that administration of a high-fat diet resulted in a markedly increased Firmicutes/Bacteroides ratio and increased Proteobacteria, Actinobacteria, and Spirochaetes; elevation of inflammatory markers in the serum and choroid tissue; and exacerbated laser-induced choroidal neovascularization and Iba1 immunostaining (a marker of microglial activation).11 Moreover, mice fed a high glycemic diet developed phenotypic features of dry AMD (retinal pigment epithelium atrophy, lipofuscin accumulation, and loss of photoreceptor cells), modified gut microbiome (enrichment of Firmicutes and Clostridia), and altered serum and urine metabolites.12 Switching mice from high to low glycemic diets, and follow-up experiments of fecal microbiota transplant from low glycemic to high glycemic mice, resulted in microbiome changes, arrest of AMD development, and reduced retinal lesions.13 Altogether, the review of Xiao et al1 elaborates on various aspects of AMD and the microbiome that help us understand the bigger picture.

Uveitis

Janetos et al14 highlight the microbiome and its role in uveitis, primarily noting that the gut microbiome affects uveitis development through molecular mimicry, imbalance of regulatory and effector T cells, increased intestinal permeability, and loss of intestinal metabolites. Similar to patients with other ocular diseases, patients with uveitis also lack microbiome diversity and have an abundance of proinflammatory bacteria such as Prevotella and Clostridia. Patients with uveitis have a decrease in butyrate-producing bacteria, such as Faecalibacterium, Blautia, Roseburia, Lachnospira, and Ruminococcus. These butyrate-producing bacteria stimulate the release of mucus to strengthen the gut epithelial barrier, and they also contribute to a beneficial
anti-inflammatory response by generating extrathymic regulatory T cells.\textsuperscript{15}

Janetos et al\textsuperscript{14} lay out the two frameworks used to study the gut microbiome and uveitis: induced and spontaneous models of experimental autoimmune uveitis. The former activates an autoimmune response against the retina by introducing an antigen, and the latter uses a transgene from a uveitis-susceptible mouse to generate reactive T cells that develop autoimmune uveitis without requiring an outside trigger.

A study using the spontaneous experimental autoimmune uveitis model showed that the microbiota composition was variable between mice with uveitis treated with broad-spectrum antibiotics versus those untreated.\textsuperscript{16} In treated mice, uveitis severity was attenuated, and \textit{Proteobacteria} and \textit{Tenericutes} predominated. In contrast, untreated mice showed an abundance of \textit{Bacteroidetes} and \textit{Firmicutes} species. Another study showed that, in an induced experimental autoimmune uveitis mouse model, oral broad-spectrum antibiotics attenuated inflammation related to uveitis, whereas intraperitoneal administration had no anti-inflammatory effect.\textsuperscript{17} Janetos et al\textsuperscript{14} aptly suggest that it is an alteration in the gut microbiome, not just antibiotics alone, that changes uveitis severity. In a spontaneous model of experimental autoimmune uveitis, individual treatment with various antibiotics did not significantly alter disease course, whereas combination treatment had a therapeutic effect.\textsuperscript{18} This review shows that the gut microbiome clearly has some effect on uveitis. However, many complexities need to be better understood to design definitive therapeutics.

**Inherited Retinal Degenerations**

Inherited retinal degenerations, a large group of disorders that are genetically determined and cannot be prevented, have also been studied through the lens of the microbiome in hopes of developing better tools to delay disease progression. In their review, Douglas et al\textsuperscript{18} focus on retinitis pigmentosa and Batten disease, both debilitating diseases without a cure. They described a mouse model of retinitis pigmentosa, in which retinal degeneration 10 (\textit{rd10}) mice were compared versus wild-type mice. At baseline, there were significant differences in diversity of the microbiota, and \textit{rd10} mice exhibited an abundance of \textit{Bacteroides caecimuris} and a lack of \textit{Rikenella}, \textit{Muribaculaceae}, \textit{Prevotellaceae} UCG-011, and \textit{Bacilli}.\textsuperscript{19} After administration of a high-fat diet for 2 to 3 weeks, there were significant alterations in microbiota diversity, with an increase in \textit{Bilophila}, \textit{Alistipes}, and \textit{Mucispirillum schaedleri}, which are correlated with gut inflammatory processes. \textit{Muribaculaceae}, involved in gut homeostasis, was decreased after administration of a high-fat diet. These microbial shifts were also accompanied by worsening of retinal degeneration in \textit{rd10} mice (as measured by decreased full-field flash electroretinogram amplitudes, optomotor response, and photoreceptor outer nuclear layer thickness). Moreover, two mice strains modeling infantile CLN1-linked and late infantile CLN2-linked forms of Batten disease were shown to have increased \textit{Erysipelotrichaceae}, and the \textit{Cln1} mice showed decreased \textit{Bacteroides} compared with wild-type mice.\textsuperscript{20} Of note, both strains showed a decrease in \textit{Lactococcus}, lactic acid–producing bacteria with known anti-inflammatory and cytoprotective properties. Interestingly, administration of acidified water in a mouse model for CLN3-associated Batten disease caused a relative increase in the abundance of \textit{Lactobacillus} and attenuated motor deficits and microglial activation in several areas of the brain.\textsuperscript{21} This finding suggests that changes in gut microbiota modify anti-inflammatory properties and strengthen the potential association between microbial composition, microbial metabolites, and retinal degeneration.

**Retinal Vascular Diseases**

Lincke et al\textsuperscript{22} undertake a nuanced review relating to the gut microbiome, vascular disease risk factors (obesity, hypertension, sedentary lifestyle, diabetes mellitus, dyslipidemia, and atherosclerosis), and retinal vascular diseases (diabetic retinopathy [DR], retinal vein occlusion, and retinal artery occlusion). Compared with healthy patients, patients with non-arteritic retinal artery occlusion showed an enrichment of \textit{Actinobacteria} (linked to development of atherosclerotic plaques), \textit{Bifidobacterium adolescentis}, \textit{Bifidobacterium bifidum}, \textit{Bacteroides stercoris}, and \textit{Faecalibacterium prausnitzii}.\textsuperscript{23} Patients with retinal artery occlusion also had enrichment of pathways involved in cholesterol metabolism. In their review, the authors highlighted that individuals with DR have compositional and functional changes in the gut microbiome compared with healthy individuals and patients with diabetes mellitus only. Compared to patients with diabetes mellitus, patients with DR had lower bacterial diversity and increased \textit{Bacteroides} with decreased \textit{Blautia} and \textit{Lactobacillus}.\textsuperscript{24}

A separate study showed an increase of \textit{Burkholderiaceae} and uncultified \textit{Burkholderiales} in patients with DR.\textsuperscript{25} Fecal samples from patients with DR displayed increased levels of arachidonic acid, hydroxyeicosatetraenoic acids, and leukotriene, which are known mediators for DR development.

Neuroprotective bile acid tauroursodeoxycholate, whose metabolism may be modulated by \textit{Firmicutes} bacteria, has been implicated in DR.\textsuperscript{26} Interestingly, retinal primary ganglion cells express the tauroursodeoxycholate receptor TGR5, and its activation by pharmacologic agents prevented DR in a mouse model. In diabetic mice, compound dietary fiber and a high-grade protein diet increased \textit{Firmicutes} and decreased \textit{Bacteroidetes}, alongside an observed decrease in hyperglycemia and insulin resistance. In a comparison of atherosclerotic patients versus healthy
control subjects, atherosclerotic patients exhibited an enrichment in Collinsella and a decrease in Eubacterium and Roseburia. Various bacteria such as Klebsiella, Prevotella, Desulfovibrio, and Parabacteroides have also been associated with hypertension. In a mouse model, fecal microbiota transplant from a hypertensive mouse to a normotensive mouse caused a shift in microbiome composition and resultant hypertension. Lincke et al also described how microbial metabolites, such as lipopolysaccharides and trimethylamine N-oxide, play a role in cardiovascular disease development with a downstream effect on retinal vascular disease. Lipopolysaccharides likely trigger neurodegeneration by activating microglia, and trimethylamine N-oxide modulates metabolic pathways that are thought to affect atherosclerosis and diabetes mellitus, although the exact mechanisms remain unclear. This review nicely illustrates the notion that dysbiosis may promote a host of vascular risk factors that have a large role in retinal vascular disease.

Retinopathy of Prematurity

In contrast to many of these ocular diseases that progress with age, ROP is a vasoproliferative disease of the retina that is the leading cause of childhood blindness worldwide. Although we tend to think about insults to the microbiome in terms of aging and lifestyle choices, the microbiome is also particularly sensitive to external factors in preterm infants. As Zhang et al describe in their review, preterm infants are more exposed to the hospital environment, antibiotics, and feeding tubes with biofilms. They are also more likely to experience intermittent hypoxia. All these factors together can cause changes in an infant’s microbiome, resulting in decreases in Firmicutes, Bifidobacterium, and Lactobacillus with increases in Clostridium and Proteobacteria.

High-risk premature infants who later developed type 1 ROP display an enrichment of Enterobacteriaceae at 28 weeks’ postmenstrual age compared with high-risk premature infants who do not develop any ROP. Importantly, Enterobacteriaceae up-regulate vascular endothelial growth factor, which is a key player in neovascularization in ROP and retinal detachment. Infants with ROP also had differences in arginine, proline, and histidine metabolism and valine, leucine, and isoleucine degradation; these changes may result in increased oxidative stress in retinal cells.

Another study found that infants with ROP exhibited significantly reduced bacterial diversity and had a significant enrichment in Staphylococcus. Zhang et al also highlight the potential relationship between ROP and insulin-like growth factor 1, an anabolic hormone involved in postnatal growth and development and vascular endothelial growth factor regulation. These shifts in microbiome interfere with tissue revascularization and vascular repair, as well as metabolism, affecting processes involved in oxidative stress, which contribute to ROP pathogenesis. Zhang et al also describe how a better understanding of the microbiome and ROP can advance screening techniques and therapeutic interventions.

Ocular Surface Microbiome

Although the aforementioned reviews all focus on the gut microbiome, Zilliox et al provide a unique commentary on the OSM. Similar to the gut microbiome, a key marker of a healthy OSM is diversity of microbes. The OSM is also sensitive to a host of factors, such as age, sex, geography, and diet. Traditional cultures of a healthy ocular surface show predominance of Staphylococcus, Cutibacterium, and Corynebacterium. However, a lot of investigation into defining and measuring the “core” bacteria of the OSM is still required.

Zilliox et al describe how variations in methodology (eg, culture-only studies versus sequencing-only studies versus combined culture and sequencing studies) result in different classifications of “core” bacteria. This review also describes the challenges of measuring the OSM, including sampling methods, topical anesthetics, concurrent use of other topical medications, contact lens wear, DNA isolation methods, and bioinformatics.

Zilliox et al also describe the relationship between the OSM and various diseases, including dry eye disease, Sjögren’s syndrome, meibomian gland dysfunction, and ocular graft-versus-host-disease, among others. Across this broad spread of diseases, many of them exhibit a reduction in bacterial diversity compared with healthy control eyes. It is thought that commensal bacteria on the ocular surface inhibit pathogenic bacteria (eg, Pseudomonas overgrowth), stimulate expression of genes involved in tear film stabilization, and act as a physical barrier to prevent pathogenic molecules from entering through the eye. Although there are improvements that can be made to better study the OSM, the insight of Zilliox et al into the OSM complements the other reviews focusing on the gut microbiome.

Conclusions

Altogether, the reviews in the Microbiome and Ocular Health thematic issue of The American Journal of Pathology provide an excellent discussion of how changes in microbial composition are associated with a wide range of ocular disease states, potential mechanisms explaining how dysbiosis affects disease progression, and innovative screening and therapeutic solutions that harness the power of the microbiome. It is our hope that this issue will encourage further advancements in understanding the powerful relationship between the microbiome and ocular disease. Given the relative ease of microbiome manipulation, we are optimistic that future therapeutic innovations
will be accessible to many and improve both their vision and quality of life.

References

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