ARE MICROBIOTA AND PROBIOTICS READY FOR USE IN OPHTHALMOLOGY?


Individualized treatments can help to improve some inflammatory conditions.

BY CARLOS VERGÉS, MD, PHD, AND VERONICA RIBAS GONZÁLEZ, MD

Recent evidence shows that microbiota play an important role in diseases such as dry eye disease (DED),[1,2] glaucoma,[3,4] uveitis,[5] and age-related macular degeneration (AMD).[6,7] Is it time to introduce this knowledge and the use of probiotics in our daily practice?

WHAT WE KNOW

The human microbiota are defined as the collection of microorganisms colonizing human body sites. *Microbiome* is a different term used in the context of genomics to refer to the collection of genes that the microbiota harbor. Many times, these two terms are used interchangeably. The microbiome is studied in metagenomics, a discipline in which researchers conduct simultaneous analysis of the DNA obtained from all these microbial communities.[8]

The systemic microbiota were characterized in 2008 and the microbiota of the ocular surface in 2011.[9,10] Much progress has been made since then in understanding the bacterial composition of the eye. Previous studies used traditional microbiology techniques to characterize the ocular surface microbiota, but culture-based techniques report only 5% of its composition.

The emergence of DNA sequencing techniques was a breakthrough, demonstrating the presence of a significantly larger number of bacteria in the composition of the microbiota. Studying marker genes, such as analysis of the 16S rRNA gene with polymerase chain reaction, researchers noticed that the presence of 12 bacterial genera was repeated in almost 95% of cases, highlighting five of them in particular: *Pseudomonas, Bradyrhizobium, Propionibacterium, Acinetobacter*, and *Corynebacterium*.

Recent studies support the presence of these microorganisms and conclude that each person has a similar and stable microbiota with some differences akin to an individual fingerprint.[11,12]

Microbial colonization takes place at birth, from the mother's microbiota in the birth canal.[13] The mucosal immune system protects the ocular surface and the conjunctiva through innate and adaptive defense mechanisms.[14]

Studies have shown that ocular surface epithelial cells can recognize and selectively respond to microbial components. The recognition of components from pathogenic bacteria induces the production of proinflammatory cytokines.[15] The lack of an inflammatory response to nonpathogenic bacteria implies a unique innate immune response of the ocular surface epithelium that supports the colonization of resident commensal microbiota.

In some situations, the innate immune system at the corneal and conjunctival epithelium can be breached. If disruption of the ocular surface occurs, microbial ligands are allowed to trigger ocular inflammation.[16,17] Additionally, alterations in the microbiota of the ocular surface have been associated with inflammatory pathologies such as DED, contact lens wear, antibiotics, uveitis, glaucoma, and infections.[16]

Epigenetics is a new field of study that focuses on the role that epigenetic changes play in the genesis of certain diseases that affect the eye.[18] Epigenetics can be described as the study of modifications in gene expression that are not due to an alteration in the primary structure of a DNA sequence. These modifications include processes such as DNA methylation and histone modification, and they can be passed down from generation to generation.

Environmental factors are also important sources of gene modulation. Because of the plasticity of the genome, adaptation to the environment is possible, resulting in the expression of different phenotypes depending on the environment to which an individual is exposed. Dysbiosis of the ocular microbiota can lead to epigenomic dysregulation, altering the innate immune tolerance and resulting in the triggering of inflammatory processes that can cause eye diseases. This has been suggested in some studies of AMD and uveitis (detailed in the next section).

RELATIONSHIPS BETWEEN MICROBIOTA AND EYE DISEASE

The characterization of intraocular microbiota in patients with AMD provides evidence supporting an infectious etiology of this disease. A high concentration of *Bacillus megaterium* has been found in soft drusen in AMD.[6]

Studies of experimental autoimmune uveitis induced in mice showed that gut microbiota can play a role in the concentration of T-cells and cytokines in both intestinal and extraintestinal

tissues and also in the activation of retina-specific T-cells that can be involved in autoimmune uveitis. Although more studies are needed, these results emphasize the potential role of microorganisms rather than autoantigens in triggering pathologic immune responses that damage the eye in uveitis models. [5,19]

Patients with type 2 diabetes have an elevated incidence of eye infections.[20] A high glucose level favors bacterial growth in the skin, on the ocular surface, and in tears, and it has the ability to alter the conjunctival bacterial microbiota.[21] At the same time, the intestinal microbiota is altered in patients with diabetes, and microorganisms and residual metabolites (short-chain fatty acids) with proinflammatory activity leak into the blood,[22] which can potentially affect the eye.

Li et al reported a reduction of *Proteobacteria* and *Acinetobacter* and the proliferation of *Bacteroidetes* on the ocular surface and an increase of *Bacteroidetes* in the intestinal flora of diabetic patients. Their study concluded that imbalances among the main species of the bacterial microbiota can alter the eye's defenses, allowing the entrance of pathogenic microorganisms and proinflammatory molecules. Other studies have indicated a high incidence of DED in patients with diabetes. The increase in T-cells and inflammatory cytokines and the decrease in goblet cells from the conjunctival epithelium is associated with the appearance of signs and symptoms of DED.[23]

DED is one of the pathologies most closely related to dysbiosis and changes in the intestinal and ocular microbiota. A significant increase in the bacterial population has been noted, and bacteria that were present only in DED, such as *Bacillus spp*. and *Klebsiella oxytoca*, have been found.[1] Fukushima et al detected an increase in conjunctival bacterial count that correlated with a decrease in goblet cells and the mucin layer, one factor that triggers DED.[24]

Microorganisms such as *Candida* and *Streptococcus* mutans increase in the saliva of patients with Sjögren syndrome, whereas *Fusobacterium nucleatum* colonies practically disappear.[25] A drastic reduction of *Faecalibacterium*, *Bacteroides*, *Parabacteroides*, and *Prevotella* and an increase in the genera *Escherichia*, *Shigella*, and *Streptococcus* occur in the intestinal microbiota of patients with Sjögren syndrome.[2] The authors concluded that "the severity of the ocular and systemic disease of [Sjögren syndrome] is inversely correlated with the microbial diversity of the microbiota." Sjögren syndrome, they said, is marked by a dysbiotic intestinal microbiome powered by a low relative abundance of commensal bacteria and a high relative abundance of potential pathogenic genera.

There is also evidence linking glaucoma to dysfunction of the eye's microbiota. The altered commensal microbiome can induce changes in cytokine signaling and complement activation, triggering an inflammatory degenerative response in the retina and optic nerve.[3] The role of the human microbiome in modulating levels of brain-derived neurotrophic factor, which has been shown to have an effect on the survival of retinal ganglion cells, has been noted in animal experimental studies.[4]

Finally, it is well known that excessive or inappropriate use of antibiotics can alter the ocular microbiota and cause serious infections owing to the growth of other pathogenic bacteria or can induce processes of autoimmune origin. The ARCANE study concluded that repeated use of ophthalmic antibiotics, specifically macrolides and fluoroquinolones, leads to a significant increase in Gram-positive species, particularly *Staphylococcus epidermidis*.[26] Repeated use of a topical antibiotic such as moxifloxacin after intravitreal injection was correlated with increased resistance to antibiotics in the ocular surface microbiota.[27]

PROBIOTICS IN OPHTHALMOLOGY

After seeing how the intestinal and eye microbiota can play important roles in some eye diseases, we wondered whether steps can be taken to improve the microbiota with the use of probiotics. Probiotics, a combination of live beneficial bacteria and yeasts that naturally live in the body, have multifactorial effects, and positive results have been reported with their use in inflammatory pathologies.[28]

Microbiota and intestinal dysbiosis could be involved in Sjögren syndrome. Studies have shown that systemic and ocular manifestations can be improved in patients with Sjögren syndrome by treating intestinal dysbiosis and improving the microbiota with dietary fiber, prebiotics, and probiotics.[29]

Chisari and colleagues studied patients with DED treated with *Bifidobacteria* and *Lactobacillus* and concluded that this type of therapy can improve DED.[30] In another study, DED treatment with probiotics that contained *Saccharomyces boulardii* MUCL 53837 and *Enterococcus faecium* LMG S-28935, administered orally or topically, mixed with artificial tears produced a noticeable improvement in patients without significant complications.[31,32]

Lactobacillus has also been studied as a probiotic. In patients with type 2 diabetes, significant decrease in low-density lipoprotein and cholesterol and better control of the disease were demonstrated.[33,34]

Further, results from a study in patients with uveitis and DED treated with IRT-5, which contains *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus reuteri*, *B bifidum*, and *Streptococcus thermophilus*, suggest that the administration of this probiotic can modulate clinical manifestations of autoimmunity in the eye.[35]

PERSONAL EXPERIENCE

We are carrying out several studies of the effects of probiotics on various eye diseases, mostly in patients with DED. We have noticed that each patient must be considered individually. When dietary pre- and probiotics are adapted to each patient, results are encouraging. Contrarily, when we prescribe a more general treatment, the results are highly variable, and, despite some symptomatic

MICROBIOTA

improvement, the effects do not reach desirable levels of significance. Management of the microbiota in this way will require collaboration between specialists and ophthalmologists, moving away from generalized treatments with little scientific basis. ■

1. Graham JE, Moore JE, Jiru X, et al. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. *Invest Ophthalmol Vis Sci*. 2007;48(12):5616-5623.
2. De Paiva CS, Jones DB, Stern ME, et al. Altered mucosal microbiome diversity and disease severity in Sjögren syndrome. *Sci Rep*. 2016;6:23561.
3. Astafurov K, Ren L, Dong CQ, Igboin C, Hyman L. Oral microbiome link to neurodegeneration in glaucoma. *PloS One*. 2014;9(9):e104416.
4. Martin KR, Quigley HA, Zack DJ, et al. Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model. *Invest Ophthalmol Vis Sci*. 2003;44(10):4357-4365.
5. Nakamura YK, Metea C, Karstens L, et al. Gut microbial alterations associated with protection from autoimmune uveitis. *Invest Ophthalmol Vis Sci*. 2016;57(8):3747-3758.
6. McHarg S, Clark SJ, Day AJ, Bishop PN. Age-related macular degeneration and the role of the complement system. *Mol Immunol*. 2015;67(1):43-50.
7. Zinkernagel M, Zysset-Burri D, Keller I, et al. Association of the intestinal microbiome with the development of neovascular age-related macular degeneration. *Sci Rep*. 2017;7:40826.
8. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. *Nutr Rev*. 2012;70(suppl 1):S38-S44.
9. Gevers KR, Petrosino JF, Huang K, et al. The Human Microbiome Project--a community resource for the healthy human microbiome. *PLoS Biology*. 2012;10(8):e1001377.
10. Dong BJ, Iovieno A, Bates B, et al. Diversity of bacteria at healthy human conjunctiva. *Invest Ophthalmol Vis Sci*. 2011;52(8):5408-5413.
11. Doan T, Akileswaran L, Andersen D, et al. Paucibacterial microbiome and resident DNA virome of the healthy conjunctiva. *Invest Ophthalmol Vis Sci*. 2016;57:5116-5126.
12. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464:59-65.
13. Wassenaar TM, Panigrahi P. Is a foetus developing in a sterile environment? *Lett Appl Microbiol*. 2014;59:572-579.
14. Knop KM. Anatomy and immunology of the ocular surface. *Chem Immunol Allergy*. 2007;92:36-49.
15. Ueta M, Kinoshita S. Innate immunity of the ocular surface. *Brain Res Bull*. 2010;81:219-228.
16. Lu LJ, Liu J. Human microbiota and ophthalmic disease. *Yale J Biol Med*. 2016;89:325-330.
17. Cavuoto KM, Galor A, Banerjee S. Anatomic characterization of the ocular surface microbiome in children. *Microorganisms*. 2019;7(8):259-269.
18. Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome. *Science*. 2009;324(5931):1190-1192.
19. Horai R, Zarate-Blades CR, Dillenburg-Pilla P, et al. Microbiota-dependent activation of an autoreactive T cell receptor provokes autoimmunity in an immunologically privileged site. *Immunity*. 2015;43:343-353.
20. Pomposelli JJ, Baxter JK 3rd, Babineau TJ, et al. Early postoperative glucose control predicts nosocomial infection rate in diabetic patients. *JPEN J Parenter Enteral Nutr*. 1998;22(2):77-81.
21. Kandarakis S A, Piperi C, Topouzis F, Papavassiliou AG. Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. *Prog Retin Eye Res*. 2014;42:85-102.
22. Chen Z, Zhu S, Xu G. Targeting gut microbiota: a potential promising therapy for diabetic kidney disease. *Am J Transl Res*. 2016;8:4009-4016.
23. Mantelli F, Argueso P. Functions of ocular surface mucins in health and disease. *Curr Opin Allergy Clin Immunol*. 2008;8:477-483.
24. Fukushima K, Sasaki I, Ogawa H, et al. Colonisation of microflora in mice: mucosal defense against luminal bacteria. *J Gastroenterol*. 1999;34:54-60.
25. Almståhl A, Wikström M, Stenberg I, Jakobsson A, Fagerberg-Mohlin B. Oral microbiota associated with hyposalivation of different origins. *Oral Microbiol Immunol*. 2003;18:1-8.
26. Dave SB, Toma HS, Kim SJ. Changes in ocular flora in eyes exposed to ophthalmic antibiotics. *Ophthalmology*. 2013;120:937-941.
27. Yin VT, Weisbrod DJ, Eng KT, et al. Antibiotic resistance of ocular surface flora with repeated use of a topical antibiotic after intravitreal injection. *JAMA Ophthalmol*. 2013;131(4):456-461.
28. Feher J, Pinter E, Kovacs I, et al. Irritable eye syndrome: neuroimmune mechanisms and benefits of selected nutrients. *Ocul Surf*. 2014;12:134-145.
29. West CE, Renz H, Jenmalm MC, et al. The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. *J Allergy Clin Immunol*. 2015;135(1):3-13.
30. Chisari G, Rampello L, Chisari EM. Microbiology synergism between tear substitutes and symbiotic treatment of patients with irritable bowel syndrome. *Acta Medica Mediterranea*. 2016;32:865-870.
31. Chisari G, Chisari EM, Borzi AM, Chisari CG. Aging eye microbiota in dry eye syndrome in patients treated with Enterococcus faecium and Saccharomyces boulardii. *Curr Clin Pharmacol*. 2017;12(2):99-105.
32. Chisari G, Chisari EM, Francaviglia A, Chisari CG. The mixture of Bifidobacterium associated with fructo-oligosaccharides reduces the damage of the ocular surface. *Clin Ter*. 2017;168(3):e181-e185.
33. Malaguarnera G, Gagliano C, Bucolo C, et al. Lipoprotein(a) serum levels in diabetic patients with retinopathy. *Biomed Res Int*. 2013:943505.
34. Li CF, Li X, Han HQ, et al. Effect of probiotics on metabolic profiles in type 2 diabetes mellitus: a meta-analysis of randomized, controlled trials. *Medicine*. 2016;95(26):e4088.
35. Kim J, Choi SH, Kim YJ, et al. Clinical effect of IRT-5 probiotics on immune modulation of autoimmunity or alloimmunity in the eye. *Nutrients*. 2017;9(11):1166.

VERONICA RIBAS GONZÁLEZ, MD

- Head, Ocular Surface Unit, Department of Ophthalmology, Hospital Universitari Dexeus, Area Oftalmológica Avanzada, Universidad Politécnica de Cataluña, Barcelona, Spain
- veroribasg@gmail.com
- Financial disclosure: None

CARLOS VERGÉS, MD, PHD

- Professor and Head, Department of Ophthalmology, Hospital Universitari Dexeus, Area Oftalmológica Avanzada, Universidad Politécnica de Cataluña, Barcelona, Spain
- Member, *CRST Europe* Editorial Board
- cverges@cverges.com
- Financial disclosure: None