The Human Microbiota: New Frontiers in Ophthalmology and Beyond

Rob Knight, PhD

Affordable DNA sequencing and advanced information processing technology give us the tools needed to study the human—including the ocular—microbiome. What we are learning sheds new light on the complex relationship between human and microbe, and is rendering former paradigms of health and disease radically incomplete.

Since its discovery, the human microbiota, the collection of commensal microorganisms that inhabits the cavities and surfaces of the human body, has been a subject of great interest to researchers. (The term “microbiota” is now preferred to the older and erroneous term microbial “flora,” which means literally “plants.”) Over the past 15 years, major advances in gene sequencing (and its plummeting cost) have enabled investigators to explore the most perplexing and fascinating questions surrounding the human microbiota (Figure 1): What species comprise it? What influences it? How does it shift over time, cultures, and continents? And, of key interest to clinicians, how might it contribute to human health and disease?

Answers to these basic questions could foreseeably lead to clinical, microbiota-modulating interventions that support a healthy balance of microorganisms in the body, as well as prevent, treat, or cure microbiota-related disease.

The Human Supraorganism

When one considers the sheer volume of organisms involved and their intimate association with human tissue, it makes sense that the microbiota should interact significantly with human physiology. There are about 20,000 protein-making genes in the human genome; by contrast, the human microbiome—the genes contained in the microbiota—is at least 100-fold larger. As many as a hundred trillion microorganisms are present in the human gut—again, up to an order of magnitude greater than the estimated number of human cells.

See INSIDE for:
Acanthamoeba Keratitis: Risk Factors, Diagnosis, and Medical Therapy
by John E. Sutphin, Jr., MD
The enormous size of the microbiome implies that a tremendous number of biochemical functions are being performed immediately adjacent to human cells, creating miniature ecosystems and habitats in every nook and cranny of the human body. And biochemical activity in the gut, and perhaps in other body sites, can release metabolic products into the bloodstream, which then circulate and exert effects throughout the body.

The human microbiome project (HMP) is an NIH-funded research effort focused on the human microbiome and its implications in human disease. This project has labeled humans as “supra-organisms,” more complex and more microbe-dependent than we ever imagined, with traits relatable to both human and microbial cells derived from a unique personal library of human and microbial genes.1

The clinical implications of this combined existence is a subject of great interest to researchers and clinicians alike, particularly as it pertains to immuno, autoimmunity, inflammation, and susceptibility to infection. We know that the human immune system is constantly attempting to discriminate between threatening and nonthreatening microbes in its midst. To be successful, it must recognize and accommodate (rather than attack) massive numbers of different bacterial antigens on the cell surfaces of resident microbes that pose no threat and, indeed, are likely to be performing useful functions like digestion, energy homeostasis, vitamin production, and maintaining barrier integrity.2

Furthermore, the immune system—and other body systems—must live peacefully with a slew of enzymes, metabolites, etc, secreted by bacteria into the shared ecosystem; and vice-versa. Thus, as with any symbiotic relationship, health depends on effective communication and both parties having the chance to live up to their end of the bargain. A review of the microbiome as it relates to inflammatory bowel disease (IBD) noted: “The human microbiome determines and defines the immune...

STATEMENT OF NEED

Ophthalmologists face numerous challenges in optimizing their competencies and clinical practices in the realm of preventing, diagnosing, and treating ocular infections and their sequelae; these challenges include:

• The widespread "off-label" use of topical ophthalmic antibiotic, to prevent and treat serious and sight-threatening infections—giving the reality that the most widely used topical antibiotics in ophthalmology have FDA approvals restricted to bacterial conjunctivitis.
• The escalating levels of multi-drug resistance in common ocular pathogens.3
• The emergence and increasing prevalence of once-atypical infections that may require diagnostic and treatment techniques relatively unfamiliar to comprehensive ophthalmologists.4
• The introduction of new and potentially more efficacious and/or safe ophthalmic anti-infectives.5
• The introduction of new and potentially more accurate diagnostic techniques for ophthalmic infections.6
• Widespread discussion over the efficacy and safety of novel or alternative delivery techniques and vehicles for prophylactic ophthalmic antibiotics (including but not limited to intracameral injection and topical mucocutaneous).7
• Increased understanding of the inflammatory damage caused by ocular infections and the best ways to prevent/alter the inflammatory reaction without fuelling the growth of pathogenic organisms.

Given the continually evolving challenges described above, Topics in Ocular Antinfectives aims to help ophthalmologists update outdated competencies and narrow gaps between actual and optimal clinical practices. As an ongoing resource, this series will support evidence-based and rational anti-infective choices across a range of ophthalmic clinical situations.

REFERENCES


FACULTY AND DISCLOSURE STATEMENTS

Nisha Acharya, MD (Faculty Advisor), is an associate professor of ophthalmology and epidemiology at the University of California, San Francisco and director of the Veits Service at the FL Proctor Foundation. She states that in the past 12 months, she has not had a financial relationship with any commercial organization that produces, markets, re-sells, or distributes healthcare goods or services consumed by or used on patients.

Natalie Ashary MD, FACS (Faculty Advisor), is professor of ophthalmology and chief of corneas and refractive surgery at the Shiley Eye Center, University of California San Diego. She states that in the past 12 months, she has not had a financial relationship with any commercial organization that produces, markets, re-sells, or distributes healthcare goods or services consumed by or used on patients.

Melissa Duvoyt, MD (Faculty Advisor), is an assistant professor of ophthalmology and chief of corneas and refractive surgery at the Sibley Eye Center, University of California San Diego. She states that in the past 12 months, she has not had a financial relationship with any commercial organization that produces, markets, re-sells, or distributes healthcare goods or services consumed by or used on patients.

John E. Surfijn, Jr., MD, is the Luther and Ardis Fry Professor and Chairman of the Department of Ophthalmology at the University of Kansas Medical Center in Kansas City, KS. He has no relevant financial interests to disclose. He states that in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, re-sells, or distributes healthcare goods or services consumed by or used on patients.

DISCLAIMER Participants have an implied responsibility to use the newly acquired information to enhance patient outcomes and professional development. The information presented in this activity is not meant to serve as a guideline for patient care. Procedures, medications, and other courses of diagnosis and treatment discussed or suggested in this activity should not be used by clinicians without evaluation of their patients’ conditions and possible contraindications or dangers in use, applicable manufacturer’s product information, and comparison with recommendations of other authorities.

COMMERCIAL SUPPORTERS This activity is supported by an unrestricted educational grant from Bausch + Lomb, Inc.
It is seen as increasingly plausible that certain diseases may stem from a disruption (whether genetically, environmentally, diet- or drug-induced) to the balance between the host immune system and the microbiota or the molecular “communication” methods on which it depends.

**Gut Sense**

Most of the research to date on links between the microbiota and human disease has focused on the gut, because the gut harbors the greatest concentration and diversity of microbes in the body. Aberrations in gut microbiota have been linked to obesity, IBD, irritable bowel syndrome, colon cancer, cirrhosis, and fatty liver disease, as well as systemic diseases including types 1 and 2 diabetes and rheumatoid arthritis. Animal studies additionally suggest associations between gut microbiota and metabolic syndrome and multiple central nervous system disorders including depression, autism, and multiple sclerosis.

These associations reveal that the effects of microbial communities can extend well beyond the physical spaces they inhabit. This may be due to the production of metabolites that travel to distant sites, communication through the immune system, and neural signaling from the gut to the brain. Gut and mouth microbes are now thought to be relevant to various cardiovascular, neurologic, and arthritic conditions.

Intestinal microbial communities may also influence certain eye diseases. Studies have shown that the genetically encoded immune dysregulation that produces both acute anterior uveitis and ankylosing spondylitis (AS) is also characteristic of some patients with IBD. Thus, the gut microbiome may be the etiologic link when autoimmune disorders coexist, such as uveitis and AS or IBD. Studies involving a novel mouse model of spontaneous uveitis revealed that autoreactive T-cells capable of breaching the blood-brain barrier and attacking the retina are activated in the gut by commensal microorganisms. The authors conclude that commensal microbes may play a far larger role than previously appreciated in the pathogenesis of autoimmune diseases.

**Microbiota and Medications**

Gut microbiota secrete enzymes that aid in digestion of dietary proteins and carbohydrates. Similarly, medications that are administered orally are subject to enzymatic digestion and catabolism by microbes in the gut, potentially releasing toxic metabolites. Even injected medications are typically processed by the liver. And, at least in mouse models, colonization with gut bacteria alters the expression of host-encoded cytochrome P450 enzymes that are involved in drug metabolism, so microbes may potentially affect the activity of drugs delivered by other mechanisms as well. The relationship between gut microbiota and drug metabolism is a subject of increasing research interest, as it represents a potential way to explain inter-patient and inter-population variation in drug efficacy, toxicity, tolerability, and dosing requirements. If the gut microbiome plays a role in how patients respond to medications, then characterizing its genes and functions may, in the future, help guide medication selection and/or dosing.

Furthermore, modulating the microbiota—by oral probiotic administration for example—might be a means by which clinicians predict and exert finer control over patients’ response to medications. Although the eye has not been studied in this way, the skin and gut have, as knowledge increases, there may come a time when simply swabbing the conjunctiva could provide information about a patient’s resident microbial populations and serve as a useful guide to topical ocular medication selection and dosing or other intervention.

**Ocular Surface Microbiome**

Based on data derived from culture-dependent methods, the ocular surface is home to a low concentration of organisms. They are of similar composition to that on the eyelid, which harbors higher numbers and has a microbial composition characteristic of other sites on the skin. Predominant organisms include coagulase-negative staphylococci, and previously appreciated in the pathogenesis of autoimmune diseases.

**CORE CONCEPTS**

- Decreased DNA sequencing costs and advanced computational capabilities create new possibilities for studying the human microbiome.
- The gut microbiome exerts influence both locally and systemically; gastrointestinal, cardiovascular, CNS, and autoimmune diseases may stem from gut dysbiosis.
- The ocular microbiome is not yet well characterized, especially in healthy subjects.
- Antibiotic use modulates the microbiome; implications are not known.
- Modifying the microbiota—with probiotics, prebiotics, microbial transplants, etc—may grow as a health and treatment strategy.

*Propionibacterium* spp., *Staphylococcus aureus*, *Micrococcus* spp., Gram-negative bacteria, and fungi in lower numbers. Dong and coworkers have characterized resident microbes on healthy conjunctiva using deep sequencing of 16S ribosomal RNA genes. They found 59 distinct genera and significant variability in the ocular surface microbiome among the four subjects. However, several genera were common to all: *Staphylococcus, Propionibacterium, Streptococcus, Pseudomonas, Bradyrhizobium, Corynebacterium, Acinetobacter, Brevundimonas, Aquabacterium, Sphingomonas, Streptophyta, and Methylbacterium*.

Interestingly, in Dong and coworkers’ study, almost one third of DNA reads (of which there were 115,003 total) were unclassifiable or represented novel bacteria. Several organisms identified are generally regarded as pathogenic (although most are from taxonomic groups that also have non-pathogenic representatives which may not be resolvable by the techniques used). An additional concern is that low-biomass samples, such as ocular swabs, are easily contaminated in the laboratory by microbes from the researchers’ skin or from...
Modulating the Microbiota

The possibility of modulating the microbiome for therapeutic purposes is just beginning to be explored. Successful use of fecal microbiota transplantation for the treatment of Clostridium difficile colitis and oral probiotic use for relief of various gastrointestinal diseases represent the tip of the iceberg.21 In ophthalmology, a pilot study of Lactobacillus acidophilus eye drops showed statistically significant reduction in inflammation and signs and symptoms of vernal keratoconjunctivitis.22 Sjogren’s disease, which increasingly appears to be caused by a confluence of genetic, age-related, and microbiota-related influences, might one day be addressed by modulating the ocular surface microbiome. Using a thrombospondin-1-deficient mouse model of Sjogren’s disease, researchers have shown that staphylococcal overgrowth on the conjunctiva may contribute to the development of Sjogren’s disease by inducing a local inflammatory response. Targeting the specific imbalance, for example by using thrombospondin-1-derived protein (which has antimicrobial properties), could, theoretically, reduce inflammation without wiping out the entire microbiota.23

Consequently, modulating the ocular microbiome requires establishing a baseline in healthy people, identifying deviations that correlate with disease, and longitudinal studies that show whether the effects of specific interventions can modify the microbiome in directions relevant for health. Technical barriers include difficulty obtaining DNA from low-biomass ocular swabs and processing the samples without contamination. However these difficulties also apply to studies of other near-sterile body sites such as the placenta, amniotic fluid, lung and brain, and methods for handling these situations are developing rapidly. The parallels from other better-studied body sites such as the gut, skin, and mouth are clear, and the cost and difficulty of DNA sequencing and data analysis are decreasing rapidly, making studies of the eye microbiome feasible for the first time.

REFERENCES


Rob Knight, PhD, is a professor of pediatrics and computer science and engineering at University of California, San Diego. He is founder and CSO of Biota, a company that uses microbial DNA for oil and gas exploration. He states that in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients. Medical writer, Noelle Lake, MD, assisted in the preparation of this article.
Acanthamoeba keratitis can cause significant ocular morbidity. Prompt diagnosis and immediate appropriate treatment are the keys to successful management of this potentially blinding disease.

Acanthamoeba keratitis (AK), a rare but potentially catastrophic corneal condition, is caused by infection with *Acanthamoeba*, a genus of protozoans that feed by engulfing bacteria and digesting them in phagocytic vacuoles. Readily found in soil and in watery environments, such as ponds, hot tubs, swimming pools, and sewage systems, the first US case of AK was reported in 1973 in a Texas rancher who had splashed contaminated water into his eye. Since then, the disease has been increasingly recognized. Because *Acanthamoeba* infections in the developed world are most often observed in contact lens wearers, AK has come to be viewed as a serious potential complication of contact lens wear.

Epidemiology and Risk Factors

The annual incidence of AK in the US is reported to be approximately 0.15 per million. In our tertiary care center in Kansas, we typically see 1 case every 3 to 4 months. Recent years have, however, seen sporadic outbreaks of AK in multiple states of the US.

Between 2003 and 2006, Chicago experienced a roughly seven-fold spike in the number of AK cases. An environmental pathogen, corneal infection typically follows exposure to contaminated water or soil. The *Acanthamoeba* outbreak in Chicago followed an attempt to reduce potential carcinogen exposure by lowering the levels of disinfectant in the city’s water supply. In the Chicago-area AK outbreak, as well as in other recently reported US outbreaks, nearly all of the affected individuals have been contact lens wearers.

In fact, the vast majority of AK cases in the US are associated with contact lens wear. The annual incidence among contact lens wearers in the US has been estimated at 1.65 to 2.01 cases per million. In an outbreak that took place in Iowa following regional flooding in 1993, contact lens use was the single best predictor of AK development.

Poor lens hygiene and activities, such as rinsing contact lenses with contaminated tap or well water, using homemade saline solutions to store and clean contacts, and swimming or showering while wearing lenses are known to significantly increase the risk of *Acanthamoeba* infection. Researchers have also linked the use of a specific contact lens solution to an epidemic of AK, which led to voluntary recall of the product. Although the contact lens care system was not contaminated, its efficacy in eradicating *Acanthamoeba* organisms appears to have been inadequate. Testing against *Acanthamoeba*, however, has not been a requirement for FDA approval of contact lens solutions, so there is still a potential for similar AK outbreaks to occur.

The Challenge of Early Diagnosis

Because humans have little natural immunity to *Acanthamoeba*, once AK is established, it can quickly become devastating. Early, aggressive treatment offers the best chance for a good visual outcome—indeed, there is clear evidence that the time between infection and diagnosis is the principal prognostic indicator. Diagnoses made in the first 4 weeks of infection are associated with significantly better visual outcomes. As the time from exposure to diagnosis increases, the chance of successful medical therapy decreases, while the odds that a corneal graft will be needed increase. Additionally, infections of longer duration are often accompanied by greater inflammation; and the presence of intense inflammatory reaction can put patients at high risk for vision-threatening complications, such as glaucoma and cataract.

While critical, early diagnosis of AK can be a challenge, as its early clinical presentation frequently resembles more
common types of keratitis. Not surprisingly, initial misdiagnosis of AK is extremely common and, in many cases, the interval between onset of the infection and diagnosis is longer than 4 weeks.

Early diagnosis requires clinicians to be aware of the subtle indicators of Acanthamoeba infection. Patients with AK usually report significant pain—pain that may seem disproportionate to other clinical findings. Typically, these patients are also extremely sensitive to light. Furthermore, an early Acanthamoeba infection (within 1 week of onset) is often confined to the epithelium, with formation of pseudodendrites. AK is, therefore, often mistaken for herpes simplex keratitis, a viral infection of the cornea that, albeit unusual, is much more common than AK. Signs to look for include ulceration, limbitis, (limbal injection and thickening), perineural infiltrates (infiltrates around corneal nerves), and, in later stages, ring infiltrates (Figures 1-3).

Diagnostic Tools
Where available, confocal microscopy is a useful first step in evaluating patients with suspected AK (Figure 4). A morphologic diagnosis can be established by visualization of characteristic double-walled Acanthamoeba cysts (Figure 5). However, because confocal microscopy samples only small parts of the cornea, its sensitivity suffers—the Acanthamoeba cysts may simply be missed—so negative confocal microscopy findings are not definitive. Rather, AK requires laboratory confirmation. In our practice, tissue examination—corneal scraping for both smears and culture—is performed routinely in suspected cases, not just for confirmation of the clinical diagnosis, but because many patients with AK have concomitant bacterial infections.

The smears are best read by an experienced pathologist. Acanthamoeba does not stain well with gram stain, but use of calcofluor white or acridine orange can help reveal amoebic cysts. Culturing Acanthamoeba requires special non-nutrient agar coated with a layer of


Escherichia coli or Enterobacter aerogenes for the Acanthamoeba to feed on. When Acanthamoeba is present, there will be little tracks across the culture plate, indicating consumption of the bacteria. Just a handful of specialty labs are equipped to culture for amebae. Most labs lack the diagnostic capability, but tissue samples can be sent to a cornea specialist who knows where to forward them.

Polymerase chain reaction (PCR) has been shown to be a sensitive diagnostic method for AK. By detecting Acanthamoeba DNA in tissue specimens, the technique can be useful in confirming a clinical or confocal microscopy diagnosis of AK. In the US, PCR is used less frequently for the diagnosis of AK than in Europe, in part because of a lack of commercially available test kits. In addition, the diagnostic value of PCR has yet to be proved—no head-to-head clinical trials have compared PCR with culture or even confocal microscopy. Neither confocal microscopy nor PCR can distinguish between viable and nonviable Acanthamoeba organisms, so even when Acanthamoeba DNA has been identified on the corneal surface, it remains possible that something other than Acanthamoeba is the causative pathogen.

The Treatment
Eradication of Acanthamoeba corneal infection requires aggressive antimicrobial treatment. The preferred agent is a topical biguanide—either chlorhexidine 0.02% or polyhexamethylene biguanide (PHMB) 0.02%—administered hourly for at least 96 hours. Once the patient shows a positive response, the agents can be slowly tapered. Both potent disinfectants, chlorhexidine and PHMB, can be found in topical ocular solutions, and PHMB is also known as a swimming pool cleaner. Both agents must be prepared by a compounding pharmacy, which may delay treatment. While many antimicrobials, including antifungal agents, can kill active, freeliving Acanthamoeba trophozoites, a much smaller number are also effective against Acanthamoeba cysts. The biguanides treat both forms of the organism. We typically begin therapy with chlorhexidine, which may be slightly more effective than PHMB.17

Diamidines—propamidine, hexamidine, and pentamidine—are also active against both Acanthamoeba trophozoites and cysts.18 But, none of these agents is available in the US as an eye drop, although some are readily available elsewhere. Newer antifungal drugs, such as caspofungin and voriconazole, are potentially useful against Acanthamoeba, including recalcitrant infections.19,20 The problem with these agents is, again,
To obtain CME credit for this activity, go to http://cme.ufl.edu/ocular

Topical treatment with antiamoebic eye drops typically lasts from 1 to 3 months. If treatment is effective, the patient should experience less pain after about 4 days. If pain persists, drug toxicity should be suspected. If the drug is withdrawn and clinical signs and symptoms improve, toxicity was likely the culprit. In general, biguanides are less toxic than the diamidines.

Alternative methods of pathogen eradication, such as freezing and irradiation, have met with little success in patients with AK and are not a part of antiamoebic therapy. *Acanthamoeba*, especially the cystic form, seems to be exceptionally resistant to freezing. Corneal cross-linking has been suggested as a new therapy for AK, but its effectiveness is still unproved.

**Corticosteroids**

Oral corticosteroids may be beneficial in cases with significant limbitis, scleritis, or uveitis; but the role of topical corticosteroids in the treatment of AK is controversial. In particular, early use of topical corticosteroids is not recommended—*Acanthamoeba* infection that is just taking hold usually responds rapidly to antimicrobial therapy.

More importantly, corticosteroids may cause worsening of the infection, if the diagnosis is mistaken. Topical corticosteroids may be useful to control inflammation, but only after the infection is brought under control. The treatment should begin with a simple dosing regimen—once or twice a day, and patients should be monitored closely. Dosage can be adjusted based on clinical response.

**Improving the Prognosis**

Visual outcomes in AK are highly correlated with the stage at which effective treatment was begun. Most patients who are diagnosed early can achieve eradication of the infection and reasonably good vision, eg, 20/40 or better. In cases where the diagnosis takes longer to establish, the failure rates for medical therapy increase. Once the infection is eradicated, surgical intervention to eliminate significant scarring caused by severe inflammation and necrosis of corneal tissue often yields good visual results. However, therapeutic keratoplasty performed to control an infection after medical therapy has failed has generally poor results.21

Despite the importance of a timely diagnosis, the reality is that many AK patients go undiagnosed for quite a long time. At least part of the problem lies in a lack of awareness. For clinicians to diagnose AK promptly, they must first know when to suspect it. Since characteristic signs, such as perineuritis, appear only rarely, any patients who present with a very painful corneal infection and a history of wearing contact lenses in a swimming pool or hot tub should trigger suspicion of AK. Still, because the clinical presentation of AK resembles that of other types of keratitis, misdiagnosis cannot be completely avoided.

With a provisional diagnosis of herpetic or bacterial keratitis, it is not wrong to initiate treatment immediately. But, if the patient fails to improve in 48 to 96 hours, the case must then be reevaluated, and the possibility of an *Acanthamoeba* infection should always be considered.

John E. Sutphin, Jr., MD, is the Luther and Ardis Fry Professor and Chairman of the Department of Ophthalmology at the University of Kansas Medical Center in Kansas City, KS. He has no relevant financial interests to disclose. He states that, in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients. Medical writer Ying Guo, MBBS, PhD, assisted in the preparation of this article.

**REFERENCES**


SUTPHIN references continue on page 8.


3. Which of the following is the most important risk factor for AK in the US?
A. Warm climate (e.g., south Florida)
B. Presence of a corneal dystrophy
C. Contact lens wear
D. Ocular surgery within the past 3 months

2. Which of the following is likely a component of the normal human ocular surface microbiota?
A. Acinetobacter
B. Helicobacter
C. Mycobacteria
D. None of the above

3. The collection of microorganisms residing in and on the body is called:
A. The microbiome
B. The microbiota
C. The parabiosis
D. An amplicon library

4. Which of the following methods is used to make a definitive diagnosis of AK?
A. Culture
B. Confoocal microscopy
C. PCR
D. All of the above

5. Based on animal and/or human studies, which of the following conditions has NOT been associated with the human microbiota?
A. Type 1 diabetes
B. Obesity
C. Depression
D. Refractive error

6. Which of the following best predicts the visual outcome of AK?
A. Particular species of Acanthamoeba responsible
B. Stage of disease at the time of diagnosis
C. Degree of inflammatory response
D. Size of corneal infiltrate

7. Microbiome research has opened the door for which of the following potential therapies?
A. Fecal microbiota transplant
B. Multiple antibiotic cocktails
C. Vaccination that targets resistance genes
D. All of the above

8. Which of the following factors have/have been associated with a recent outbreak of AK in the US?
A. Reduced disinfectant level in domestic water supply
B. Inadequate contact lens disinfection
C. Exposure to contaminated soil
D. A and B

9. Which of the following is an effective treatment for Acanthamoeba cysts?
A. Corticosteroids
B. Diamidines
C. Freezing
D. Irradiation

10. According to the studies cited in the article by Knight, the pathophysiology of autoimmune uveitis may be linked to which of the following?
A. Long-term antibiotic exposure
B. Increased ocular surface biodiversity
C. Autoimmune T-cells activated in the gut
D. All of the above

EVALUATION:
1=Poor 2=Fair 3=Satisfactory 4=Good 5=Outstanding

11. Extent to which the activity met the identified:
Objective 1: 1 2 3 4 5
Objective 2: 1 2 3 4 5
Objective 3: 1 2 3 4 5
Objective 4: 1 2 3 4 5

12. Rate the overall effectiveness of how the activity:
Related to my practice: 1 2 3 4 5
Will influence how I practice: 1 2 3 4 5
Will help me improve patient care: 1 2 3 4 5
Stimulated my intellectual curiosity: 1 2 3 4 5
Overall quality of material: 1 2 3 4 5
Overall met my expectations: 1 2 3 4 5
Avoided commercial bias/influence: 1 2 3 4 5

13. Will the information presented cause you to make any changes in your practice? Yes No

14. If yes, please describe:

15. How committed are you to making these changes? 1 2 3 4 5

16. Are future activities on this topic important to you? Yes No

If you wish to receive credit for this activity, please fill in the following information. Retain a copy for your records.

PLEASE PRINT CLEARLY

FIRST NAME LAST NAME DEGREE

ORGANIZATION/INSTITUTE

ADDRESS LINE 1

ADDRESS LINE 2

CITY STATE ZIP

PHONE FAX