News in Review

COMMENTARY AND PERSPECTIVE

Bacterium Protects Against Other Microbes

THE MUCOSAL SURFACE OF A HEAL-thy eye is awash in a stew of antimicrobial molecules and immune cells. But, under homeostatic conditions, the bacterial species *Corynebacterium mastitidis* (*C. mast*) can thrive there harmlessly while also boosting the eye's immunological defenses against other infections, researchers have discovered.

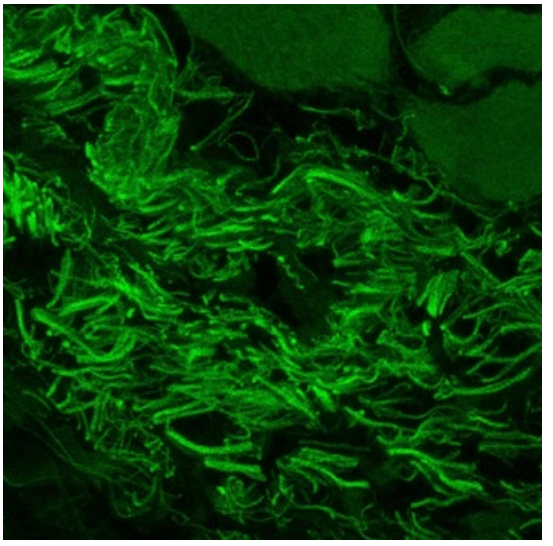
In a series of mouse experiments, a team of scientists at the National Eye Institute (NEI) found that *C. mast* induced T-cells in the ocular mucosa to produce interleukin-17 (IL-17), which controls the local production of antimicrobial molecules. In turn, this prevented invasive surface infections of *Candida albicans* and *Pseudomonas aeruginosa*.[1]

Ocular microbiome. There has been increasing recognition in recent years that commensal bacteria—the "microbiome"—play important roles in localized immune processes of the skin, gut, and other organ systems, said coauthor Rachel R. Caspi, PhD. But there was some doubt that commensal bacteria could survive on the ocular surface, she said.

"There was really no consensus that anything could live there, because the surface of the eye is highly antibacterial. We have lysozyme in tears. We have antibacterial peptides and other substances. And neutrophils come out onto the surface of the eye and patrol the eye for pathogens," she said. "What we were able to find is that the bacterium that we have identified actually lives on the ocular surface for the long term."

Persistence on surface. In mice, the NEI researchers found, *C. mast* is transmitted from mother to pup, but it is not passed between adult animals. This supports the notion that *C. mast* actively colonizes the tissue and is not continually reinoculated from the skin, said Anthony St. Leger, PhD, lead author of the study.

"The *C. mast* colonizes the eye very early in life, before the immune system has a chance to mature. Despite the bacterium stimulating an immune response, somehow it has evolved a way to avoid being eradicated from the eye," he said.



IMMUNE RESPONSE. C. mast, *found on the murine ocular surface and fluorescently labeled in this image, stimulates a beneficial immune response in the ocular mucosa—and is not eradicated from the eye.*

Next steps. In addition to determining how the bacteria persist, the researchers plan to investigate the impacts of antibiotic medications on the commensal system. "We treated the mice with antibiotics and it effectively killed the bug, but it suppressed that immune response as well. So I think our study highlights the need to better understand how topical and systemic antibiotics manipulate the ocular microbiome," Dr. St. Leger said.

"For the moment, we've just stopped at the point of finding that taking this bacterium away leaves the eye open to pathogenic infections," Dr. Caspi said. "We have not gone beyond that to try to see if we leave these mice alone now if this bacterium is going to come back, and maybe even become antibiotic-resistant."

From mice to people. It is too early to know if *C. mast* or another microbe might also function as a commensal in the human eye. However, it is likely, as *Corynebacterium* species are routinely found in conjunctival swabs of humans, said coauthor H. Nida Sen, MD, MHSc.

"I think the relevance to the human ocular surface has to be established

National Eye Institute

first," Dr. Sen said. "But conceptually, as a [research] group, we are interested in what this might mean for human inflammatory diseases or ocular surface diseases in general. Manipulation of the microbiome has the potential to shift the paradigm in treatment and prevention of disease."

For instance, perhaps contact lens–related infections could eventually be treated with commensal eyedrops that boost the localized immune system, Dr. Caspi said. "We probably don't want to be instilling live bacteria into the eye. So we would want to test preparations—either an attenuatation or, more simply, an extract from the bacterium—for their ability to replace the whole entire bug. The goal would be to mimic a commensal bacterium, but it would be under our control," she said.

—Linda Roach

1 St Leger AJ et al. *Immunity*. 2017;47(1):148-158.e5.

Relevant financial disclosures—Drs. Caspi, Sen, and St. Leger: None.

RETINA

Physical Activity and AMD Risk

IN A REMINDER OF THE IMPACT OF lifestyle factors on disease development, researchers conducted a meta-analysis of studies on physical activity and age-related macular degeneration (AMD). They found that a more active lifestyle was independently associated with lower odds of both the early and late forms of the disease.[1]

Much of the literature published to date on AMD and exercise has either produced conflicting results or has found "only small effects with unclear overall significance," said Robert P. Finger, MBBS, PhD, at the University of Bonn in Bonn, Germany. "We have now demonstrated that there is a clear association and [confirmed] that it might be worthwhile to assess physical activity in longitudinal studies of AMD."

Findings. The authors identified 620 eligible published studies, 9 of which were selected for analysis. Because of the higher prevalence of AMD among whites and the role of genetics on disease development, studies with non-white populations were excluded.

Positive effect. To account for study variations in physical assessment, participants were broadly classified as either sedentary or active.

Even a modest amount of exercise—3 hours per week of low-to-moderate activity—conferred benefits, the researchers found. The greatest reduction in the odds of having AMD was seen with the late form of the disease; active participants were 41% less likely than were their sedentary counterparts to have late AMD. With respect to early AMD, the benefit was much more modest: Active participants were 8% less likely to have early AMD compared to their sedentary counterparts.

Limitations and nuances. "The studies we pooled were cross-sectional; we still lack good longitudinal data on this research question," Dr. Finger said.

Moreover, he said, the studies mea-

IMAGING

FLIO Images Earliest Retinal Changes

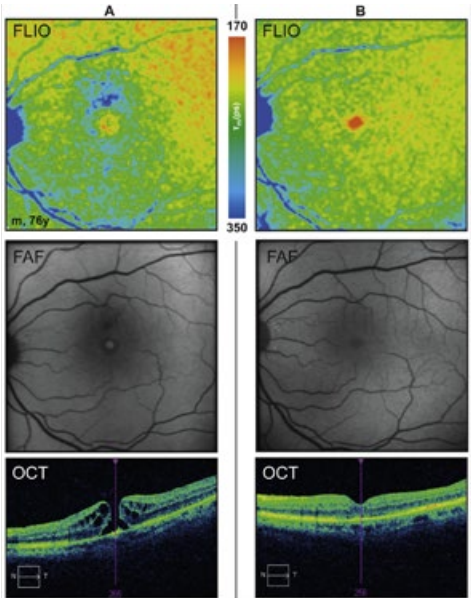
FLUORESCENCE LIFETIME IMAGING ophthalmoscopy (FLIO), a novel imaging modality that reveals specific patterns in almost any fundus disorder, may one day serve as a tool for visualizing early retinal changes in myriad retinal disorders.

While the role of FLIO is still evolving, researchers expect FLIO to be "a cornerstone for retinal imaging in the future," said Martin S. Zinkernagel, MD, PhD, at University Hospital Bern in Bern, Switzerland, and coauthor of a review of FLIO.[1] In proof-of-concept studies, he said, "We and other groups have provided [evidence] that FLIO can provide unique information to complement that [which is] obtained through other imaging modalities."

What is FLIO? Like conventional fundus autofluorescence (FAF), FLIO is mainly a qualitative imaging procedure. (It relies on a modified Heidelberg Spectralis platform, which is not yet a standard product.) In contrast, unlike FAF, FLIO can provide quantitative analysis by measuring the fluorescence lifetime of fluorophores.

In other words, FLIO is a tool that tells how long any given retinal fluorophore glows after excitation with a laser pulse. This span of time is known as fluorescence lifetimes.

Pilot studies. Comparisons of mean lifetimes of diseased eyes to healthy controls have revealed FLIO's potential for monitoring disease progression and therapeutic outcomes, Dr. Zinkernagel said. He added that FLIO has provided information on potential markers for disease progression in retinal hereditary disorders and macular degeneration, suggesting it may be used in clinical trials on gene therapy for these diseases.



FLUORESCENT LIFETIMES. *Macular hole, before surgery (A) and 3 months after successful closure of the hole (B).*

In addition, earlier this year, researchers reported using FLIO to better understand the pathogenesis of drusen in AMD. They found that

sured physical activity using questionnaires. "We now have a much more precise way to measure physical activity using accelerometers. Data from the NHANES (National Health and Nutrition Examination Survey) have demonstrated that these can be successfully used in large-scale studies and that the magnitude of the association between amount of physical activity and AMD might be stronger than what we found in our meta-analysis."

Ongoing research. Dr. Finger's team will continue to follow this avenue of study, he said. "We are building up a cohort of patients with early and intermediate AMD, in which we will assess activity via accelerometers." In addition, he said, the Rheinland Study—a prospective cohort study based in Bonn that is examining aging of the brain and the eye—is including assessments of physical activity. *—Jean Shaw*

1 McGuiness MB et al. *Am J Ophthalmol.* 2017; 180:29-38.

Relevant financial disclosures—Dr. Finger: None.

soft drusen associated with AMD progression showed longer fluorescence decays than did hard drusen. Thus, they hypothesized, FLIO might give information about the individual risk for the development of late AMD.[2]

Looking forward. Although Dr. Zinkernagel predicted that FLIO will become an important diagnostic technology, its precise use is yet to be revealed. "Whether it will be used in an academic setting or by ophthalmologists in practice is difficult to predict based on the current knowledge." *—Miriam Karmel*

1 Dysli C et al. *Prog Retin Eye Res.* Published online June 30, 2017.
2 Sauer L et al. Time-resolved fundus autofluorescence in dry AMD. Presented at: ARVO; May 9, 2017; Baltimore.

Relevant financial disclosures: Dr. Zinkernagel—National Cancer Institute/National Institutes of Health: S; Swiss National Science Foundation: S.

GENETICS

Genetic Testing: What to Order

SOMETIMES YOU CAN HAVE TOO much information, as a molecular investigation of families with inherited retinal disease demonstrates.[1] This retrospective analysis identified disease-causing genotypes in 760 of the 1,000 consecutive families treated by a single clinician, a sensitivity well beyond the 5% chance of a molecular diagnosis when the field was young.

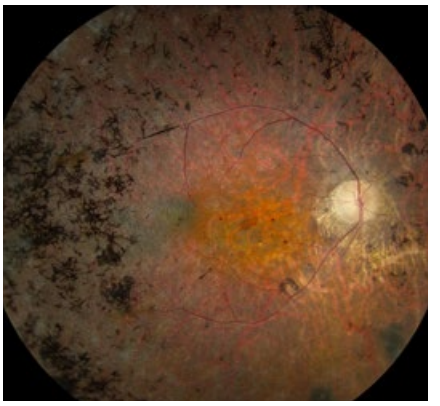
Advances in genome sequencing pose a new set of challenges to clinicians, said Edwin M. Stone, MD, PhD, at the University of Iowa Stephen A. Wynn Institute for Vision Research in Iowa City. There's a common misconception that as diagnostic modalities evolve, the need for good clinical skills diminishes, he said. In fact, the opposite is true.

"Good clinical skills are more important than ever for arriving at a correct molecular diagnosis," Dr. Stone said. "Because of the large amount of noise in each person's genome, one needs a quite focused clinical hypothesis to obtain a statistically significant result from a broad genetic test like whole exome sequencing."

Study specifics. While more than 300 genes are currently known to cause inherited retinal disorders, only 104 genes were observed in this study population. Of those, 13 genes were responsible for nearly half of disease in all families (497 families).

The researchers compared 2 testing strategies: 1) Whole exome sequencing, a single test that can examine hundreds of retinal disease–causing genes at once; and 2) tiered testing, which relies on a good clinical diagnosis to direct the molecular testing. The latter approach narrows the number of genes under consideration, thereby increasing the statistical significance of the results.

Accuracy. The results showed that the tiered testing strategy was 6.1% more sensitive than whole exome sequencing alone. It also resulted in a much lower false genotype rate (FGR). "All clinical tests have some false positives, and molecular tests are no exception," said Dr. Stone. The study showed the likelihood of observing a plausible disease-causing "result" purely by chance to be 128%, if one tested 300 genes at a time, as current retinal disease sequencing panels can do. Using a tiered approach, the FGR fell below 5%.



RETINITIS PIGMENTOSA. *A 64-year-old male with* USH2A*-associated RP. Note the characteristic lacy black intraretinal pigment, narrowed arterioles, and slightly pale optic disc.*

Cost. The study also found that a tiered approach was 17.7% less expensive than whole exome sequencing. The latter cost $1,200 per patient. In contrast, customized testing based on clinical findings cost $990 on average.

However, cost is not the reason Dr. Stone advocates a refined testing strategy. "The main value of the pretest hypothesis is to reduce the number of false positives and thereby increase the statistical significance of the results."

Should you order testing? Retina specialists could use the classification system reported in this study directly, said Dr. Stone. Other clinicians will have to customize it a bit for their practices. All physicians should try to establish the narrowest possible clinical diagnosis before performing a molecular test, he said. *—Miriam Karmel*

1 Stone EM et al. *Ophthalmology.* 2017;124(9): 1314-1331.

Relevant financial disclosures—Dr. Stone: None.

See the financial disclosure key, page 10. **For full disclosures,** including category descriptions, view this News in Review at aao.org/eyenet.