Gut check: Mapping the vast, vital world of microflora

Michail was inspired to conduct research on the gut microflora to explore new and better ways to help children battling a variety of gastrointestinal disorders.

The human body, in all of its breathtaking complexity, has inspired and mystified artists and scientists throughout history—and aspects of its form and function defy complete understanding even today.

Within our bodies, however, exists an even vaster, largely unexplored world that may provide answers to many of our most puzzling and persistent questions.

This is the realm of intestinal bacteria—called microbiota or microflora—the trillions of microorganisms that live in our digestive tracts and outnumber the body's own cells by a ratio of 10 to one. A pair of researchers at the medical school are investigating this uncharted territory in hopes of expanding basic biological knowledge and discovering more effective medical treatments for patients.

Sonia Michail, M.D., associate professor of pediatrics, and Oleg Paliy, Ph.D., assistant professor of biochemistry and molecular biology, are collaborating on a series of projects that focus on the role of microflora in gastrointestinal disorders of children. Their first two studies, already underway, are both funded by grants from the National Institutes of Health (NIH) through the National Center for Complementary and Alternative Medicine. The studies were designed to bridge the gap between basic and clinical research.

"Our hope is to take things from the bench and help children at the bedside," Michail said, a shared goal that led the physician and the scientist to embrace the idea of collaboration.

"Our Ph.D. colleagues do a much better job about basic research in general," she explained, "but I feel we're the connection to the patients we see."

A fascinating field rich with promise

As a pediatric gastroenterologist, Michail works with dozens of young patients each week who battle conditions such as Crohn's disease, irritable bowel syndrome, and childhood obesity.

With many patients, Michail said, "you know how to take care of them, and the medicine works very well, but the ones who have limited options are the ones we need to work a little harder for."

The promise of better methods of treatment or prevention strongly appealed to her, but the potential benefits of microflora research extend well beyond her specialty.

Each new study published in this growing area of inquiry, she said, provides more evidence "that would suggest that the organisms we harbor in our gut decide for us whether we get sick or stay healthy."

In addition to gastrointestinal disorders, intestinal microbiota have already been linked to some allergic skin disorders, respiratory problems, and infections of the urinary and reproductive systems.

"And those are just skimming the surface," Michail said. New connections between microflora and various disorders are announced so frequently, she said, that "it won't be surprising if any disease is going to be somewhat related to the microflora… The sky is the limit."

Still more exciting are recent reports indicating that simply altering the gut microbes can produce dramatic changes in the host. Studies have shown that lean and obese mice possess markedly different microflora, and transferring microflora from obese to lean mice causes the recipients to become obese. This pattern seems to hold true in humans as well, based on research showing that obese and lean adults have different microflora, and that as obese individuals become thinner, their microflora change to resemble those of leaner people.

"What people found was that in obese animals, their intestinal microbes were too good at extracting extra energy from the diet," Paliy said, "and this extra energy might have led to weight gain."

Based on these astonishing findings, Michail and Paliy wondered if similar results might be possible for children.

Paliy poses with the custom microarray—a chip with many thousands of oligonucleotide probes designed to test for the presence of selected DNA fragments—he and Michail developed to survey the gut microbiota.

Breaking new ground

Paliy and Michail's first NIH grant funded research to compare microflora from healthy children to those of patients with irritable bowel syndrome. Right from the outset, though, they confronted some daunting challenges.

"This is unexplored territory in pediatrics," Michail said. "It is completely new for kids. It's exciting, but at the same time, we're learning a lot as we go."

"Most of the studies of intestinal bacteria have been carried out in the adult population," Paliy explained. "Thus, we cannot tell how much of what we currently know about microbiota holds true for children."

Before they could begin analyzing the role of microbiota in specific diseases, they had to solve a more basic problem: finding a way to identify the microbes and study their activity.

Of the hundreds of bacterial species that make up the majority of the microflora, most are obligate anaerobes, or organisms that die in the presence of oxygen.

"At least 90 percent of them cannot be cultured," Michail said. "The traditional way of looking at the bacteria would not be helpful in our situation."

Rather than growing and observing bacterial samples in a controlled laboratory environment, Paliy and Michail had to rely on more specialized methods. Drawing on Paliy's expertise, they developed a new tool to catalogue intestinal bacteria and document their activity by examining the DNA from bacterial cells directly.

This approach had several advantages. First, it would work even for bacteria unable to survive or multiply outside of the intestine. Second, the tool could be used to profile stool samples, eliminating the need for a patient biopsy. Finally, it offered a very efficient way to study nearly 800 species of bacteria using a single test.

Paliy achieved these advantages by using a custom microarray—a chip containing tens of thousands of microscopic oligonucleotide probes that can each test for the presence of specific fragments of DNA.

"Our labs have put a lot of effort, time, and expense into validating this microarray," Paliy said. "Before using this tool on human fecal samples we had to make sure that it functions correctly when tested with known bacterial DNA. The medical school was very helpful in the initial stages of our collaboration by providing funds to pay for the development and testing of the microarray. Without such help, we wouldn't have been able to obtain further NIH funding."

After validating the tool, they studied a small number of samples from healthy children and adults in a pilot study.

Right away, Michail said, "We noticed some significant differences between the two populations. Our next step is to expand our data with more subject numbers."

"It appears that older children differ from adults in the relative amounts of various types of bacteria they have," Paliy said. "This is something that has not yet been recognized in the field."

Paliy and Michail then enlisted the aid of Nicholas Reo, Ph.D., professor of biochemistry and molecular biology and director of the school's Nuclear Magnetic Resonance (NMR) Laboratory. Using NMR spectroscopy, which measures the response of cellular nuclei to magnetic fields, Reo was able to help his colleagues collect data on the metabolic function of the microbiota.

Ancient medicine on the cutting edge

While the current investigation is exploring intriguing new territory in terms of basic science, Michail's goal is to translate this knowledge into tangible benefits for patients.

"The whole idea is not just to study the microflora—the names of the bacteria and what they're doing," she said. "It's how to ultimately manipulate [them] to the advantage of the child."

To that end, Paliy and Michail's second NIH grant funds a study of probiotics, or live microbes that patients can consume to encourage the development of health-promoting microflora. While this idea may sound exotic, probiotics are commercially available as dietary supplements and are included in some foods, such as specialty yogurts and beverages. They are also hardly newcomers to the world of medicine.

"Probiotics were talked about in Biblical times" and used even earlier, Michail said. "We're just beginning to understand how they work and how to apply them."

During a post-residency fellowship in pediatric gastroenterology and nutrition at Creighton University in Omaha, Nebraska, Michail said, "my mentors were interested in probiotics. I did most of my basic research training as a fellow in this area."

The leap from understanding processes at work among the microflora to influencing those processes with probiotics is a very feasible and appealing one, she believes.

"For example," she said, "we found an organism in the gut of healthy children that children with inflammatory bowel disease (IBD) do not have. That particular organism could be a good example of a probiotic that could be developed to help patients with IBD."

A future full of possibility

In addition to attempting to replicate their results with a larger group of patients, Paliy and Michail have planned a number of follow-up studies and are pursuing funding to investigate the role of microflora in conditions such as childhood obesity and Crohn's disease. They are confident about the direction and prospects of their work because of its strong potential for profound patient benefits.

"If you take obesity as an example," Michail said, "if you can modify the microflora before the child becomes obese by simple things related to diet or supplements, then you are more likely to prevent a national epidemic that costs the country billions of dollars and causes significant health and mental problems."

After studying differences in the microflora of obese and lean children, Paliy and Michail hope to measure the impact of a particular prebiotic—a non-digestible carbohydrate that nourishes helpful bacteria inside the intestine and reduces microbes linked with obesity.

"We think that may be one easy and effective way of stopping overweight children from becoming obese," Michail said.

Paliy and Michail have begun sharing their initial results, which have attracted a lot of attention. A paper describing the development and validation of the custom microarray was published in the June issue of the journal *Applied and Environmental Microbiology.* They have also presented their findings at a number of conferences, and Michail said the response has been "overwhelming."

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"People are asking to collaborate, asking questions," she said, "and we've been invited to begin some new projects."

Based on their initial success and numerous possibilities for moving forward, Paliy and Michail plan to continue their remarkably fruitful partnership—an arrangement they also enthusiastically recommend to their colleagues.

"It would be very satisfying to see more research happen on the clinical side," Michail said, "and more cooperation between the clinical faculty and the research faculty."

"I think both myself and Dr. Michail realize that what we are doing together as a team, we would not be able to accomplish on our own," Paliy agreed. VS

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