
Microbes Within Us

Scientists
Investigate
How the
Microbiome
Influences
Human Health

By Sharon Tregaskis

Photographs by Jörg Meyer

Anyone who has ever announced a pregnancy knows how freely others offer advice on sleeping habits, feeding, and other challenges that follow birth. But P&S bacteria researcher Yiping Han, PhD, offers a tip rarely included in the free-flowing advice: See a dentist and get serious about brushing, flossing, and rinsing with an alcohol-free mouthwash.

Her advice goes further: Optimize dental health in the months before you start trying to conceive to boost the baby's prospects for a healthy birth weight and full gestational age. Should an infection and swollen, bleeding gums simultaneously strike during pregnancy, says Dr. Han, see a doctor.

The advice emerges from more than 15 years in which Dr. Han's research has investigated the role of the oral microbiome in adverse pregnancy outcomes—miscarriage, preterm labor, low birth weight, and neonatal sepsis. Much of that work has focused on *Fusobacterium nucleatum*, a tubular bacterium implicated in periodontal disease and also associated with appendicitis, rheumatoid arthritis, Alzheimer's disease, and other maladies.

"There's a saying in the dental field that the mouth is the gateway to your health," says Dr. Han, also a faculty member in Columbia's College of Dental Medicine. "I think there's some justification to that."

Trillions of bacteria, fungi, and viruses populate our skin, sinuses, lungs, guts, and urinary and reproductive tracts. The oral cavity alone is home to between 700 and 1,000 species of microorganisms, which occupy such distinct habitats as the teeth, tongue, hard palate, soft palate, tonsils, and the "gingival sulcus," where teeth meet gum tissue. So many billions of beings comprise the human microbiome that scientists now estimate that their genes outnumber our own by a factor of 100 to 1. "If you add up all of the microbes in the gut, they weigh more than the brain in terms of physical mass," says biologist Harris Wang, PhD, assistant professor of systems biology. "They outnumber human cells 10 to 1. They constitute a very important area of the normal human physiology."

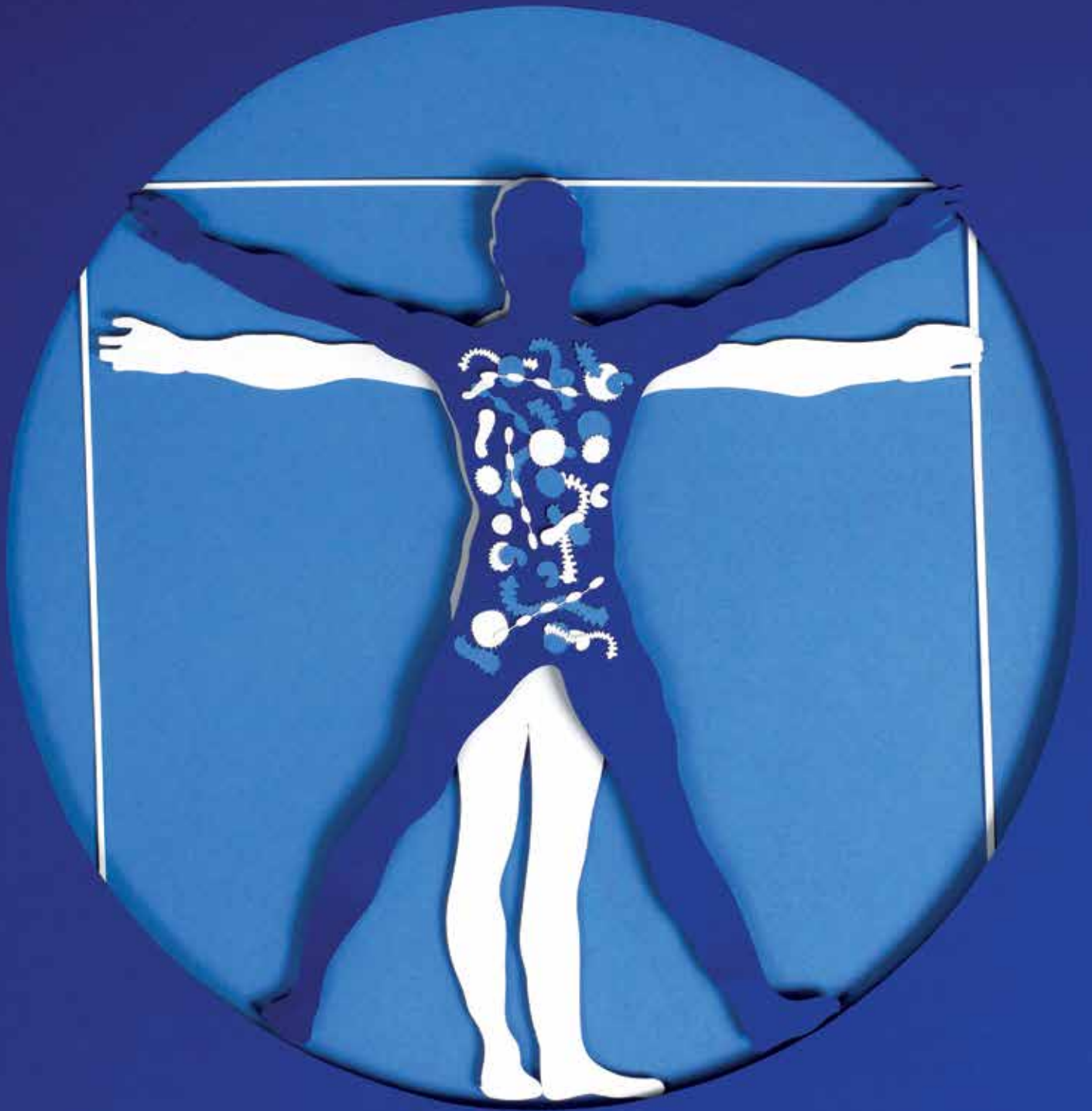
Don't make the mistake of imagining those microbes as mere bit players, says internationally renowned virus hunter W. Ian Lipkin, MD, who has joint appointments in P&S and the

Mailman School of Public Health. "We interact with them continuously and they mold who we are and what we will become. They've played important roles in evolution and they play important roles in everyday life—from how the immune system is tuned to how we respond to our environment and how we digest food."

Early research in the field—including the NIH's Human Microbiome Project, which launched in 2007—focused on naming and describing the species common to the human body. Dr. Lipkin, featured in a video as part of the American Museum of Natural History exhibition "The Secret World Inside of You," has been a leader in such efforts for more than three decades. "We have a symbiotic, or at least mutually respectful, relationship with our microbiome," he says. "It's when things get out of balance that we have difficulties—the *dis-ease* we perceive as disease."

Dr. Lipkin and Dr. Han are among a growing cadre of P&S investigators exploring what constitutes balance within the microbiome and how the many species involved maintain homeostasis with us, their human hosts. By getting a better handle on how the whole system functions, they hope to identify interventions that can promote health and prevent disease. "What I have been pushing for the last few years is for us to create an effort that moves beyond description," says Microbiology & Immunology Chair Sankar Ghosh, PhD, "to start exploring the mechanisms by which the microbiome influences physiological processes."

That effort extends from basic science to clinical applications, from new methods for microbial analysis to classic culturing. In Dr. Ghosh's lab, a team seeks to reveal how receptors within the immune system monitor the microbiome and the mechanisms by which our bodies respond briskly and aggressively to potentially dangerous species while giving beneficial species a pass. This year, Harris Wang received more than \$4 million in awards from the NIH, the Defense Advanced Research Projects Agency, and the Office of Naval Research to develop computational techniques to speed microbial discovery and genomic engineering strategies to unlock the secrets of microbes that scientists have not yet been able to culture in the lab. The projects include collaboration with



Ivaylo Ivanov, PhD, assistant professor of microbiology & immunology, on engineering commensals involved in inflammation.

In a project funded by the NIH with a five-year, \$2 million award, Dr. Han has partnered with Timothy Wang, MD, chief of digestive and liver diseases, to explore the role of *F. nucleatum* in colorectal cancer. Dr. Lipkin combines novel molecular diagnostic innovations with epidemiology and conventional clinical data to investigate the microbial hit-and-run events that seem to trigger such conditions as autism and chronic fatigue. Hepatologist Elizabeth C. Verna, MD, has begun monitoring the gut microbiome of people with liver disease, looking for clues to optimize post-transplant recovery.

An Ecological Mindset

When it comes to interactions between microbes and their human hosts, researchers sound rather like park rangers discussing how fire, drought, and myriad other environmental conditions can weight the scales in favor of one species or another. It's all about the web that holds the species together, their fates inextricably linked. "It's important to think

about bacteria, fungi, viruses, and human cells as existing in equilibrium," says Dr. Lipkin. "If equilibrium is maintained, we have health."

Like the keystone at the crown of a Roman arch, commensal species serve as vital placeholders. Take, for example, the commensal oral microbe *F. nucleatum*, the subject of Dr. Han's research and a building block of dental plaque. "Oral plaque consists of many, many bacteria—hundreds of species," she says. "They don't all just pile up randomly; they're very organized." Early colonizers bind to the protein layer that coats the tooth surface, creating crags and crevices for the secondary colonizers—*F. nucleatum* and its ilk. Late colonizers bind to the *Fusobacterium*. Without the bacteria, we would be spared tooth decay triggered by the acidic byproduct of bacterial metabolism but would be at risk of attack from more dangerous microbes. "The commensal bacteria occupy the sites in the host so that foreign pathogens—the exogenous pathogens—cannot colonize," says Dr. Han. "They build colonization resistance."

Imagine the give and take among microbes as something akin to international commerce, suggests Harris Wang, whose paper, "An Economic

Framework of Microbial Trade," appeared in PLOS One in July 2015. "A large fraction of microbial life on Earth exists in complex communities where metabolic exchange is vital," he and his co-authors wrote. "Microbes trade essential resources to promote their own growth in an analogous way to countries that exchange goods in modern economic markets." Extending the metaphor, the collaborators developed a model of microbial population dynamics based on the economic theory of general equilibrium. "Our model suggests that microbial communities can grow faster when species are unable to produce essential resources that are obtained through trade," they wrote, "thereby promoting metabolic specialization and increased intercellular exchange. Furthermore, we find that species engaged in trade exhibit a fundamental trade-off between growth rate and relative population abundance, and that different environments that put greater pressure on group selection versus individual selection will promote varying strategies along this growth-abundance spectrum."

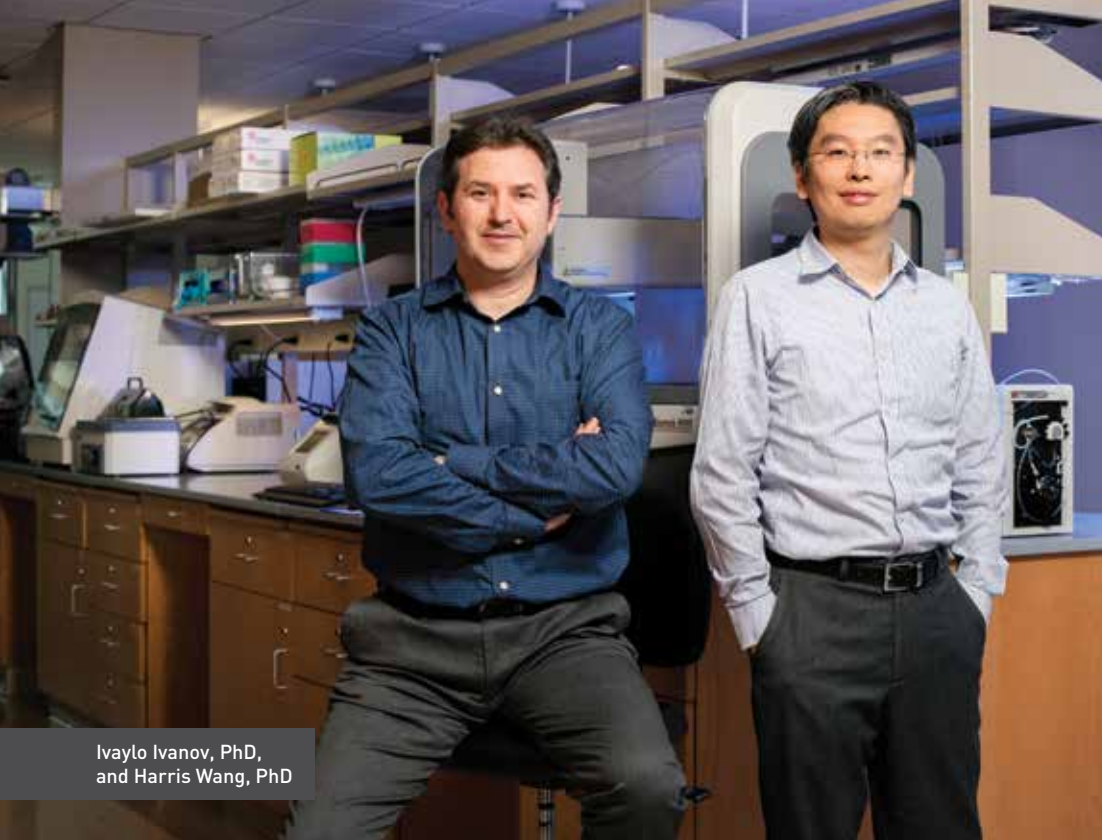
Imagine, then, how easily a commensal within the human habitat can become a pathogen. Take, for example, the fungus *Candida*, a standard fea-



W. Ian Lipkin, MD



Sankar Ghosh, PhD



Ivaylo Ivanov, PhD,
and Harris Wang, PhD

ture of the human microbiome. Typically, our immune system keeps its population in check. But when the system gets disrupted—as with AIDS or in the aftermath of intensive antibiotic treatment—oral thrush or a yeast infection emerges. Likewise, *E. nucleatum* is ubiquitous within the oral cavity but typically held in check by a robust immune system abetted by regular brushing and flossing. “Everybody has *Fusobacterium*, but not everybody has periodontal disease,” says Dr. Han. “Under normal, healthy conditions, this bacterium can exist in our oral cavity without causing much harm. What kind of role the bacterium plays depends on the host ecological environment.”

The human immune system factors heavily among the environmental influences on the microbiome, and vice versa. Over the past five years, Dr. Ivanov has investigated how relationships between commensal microbes and their host regulate and modulate the immune system. Using a combination of genomic techniques and studies conducted in a germ-free facility with specially engineered mice, his team has elucidated the molecular communion between segmented filamentous bacteria, an intestinal commensal, and the leukocytes known as Th17 cells. Known to contribute to host defense against extracellular pathogens, Th17 cells also have been implicated in the pathogenesis of multiple inflammatory and autoimmune disorders. In 2009, Cell published Dr. Ivanov’s discovery that colonization with segmented filamentous bacteria spurs Th17

cell induction, one of the first direct examples of a specific commensal modulating intestinal immune function. “It was a huge advance,” says Dr. Ghosh. “It took the microbiome down from trillions of bacteria and thousands of different species to one bacteria and from many physiological processes to the production of one cell.”

In 2012, Dr. Ivanov was named a Pew Scholar in the Biomedical Sciences and in 2013 he received a senior research award from the Crohn’s and Colitis Foundation of America to investigate how commensal microbes battle inflammatory bowel diseases. In March his project, “Keeping a Healthy Gut: Commensal Bacteria Know-Hows,” earned Dr. Ivanov a 2016 Schaefer Research Scholar award—\$50,000 in discretionary funds and \$200,000 in direct costs. Such investigations of the mechanisms by which key commensal species regulate host immunity are sure to reveal new ways of treating immune diseases, says the scientist.

Good Bug, Bad Bug

Healthy and unhealthy guts alike feature *Helicobacter pylori*, a common stomach bacterium whose population takes its cues from conditions within each individual’s digestive tract. Once vilified as a pathogen for its role in stomach ulcers, the helical microbe gets more complicated with each clue investigators eke from their studies. Timothy Wang’s research, for example, has revealed that long-term exposure to *H. pylori* triggers atrophy

of the stomach’s acid-secreting cells, with a concomitant rise in pH, which ultimately makes the stomach inhospitable to *H. pylori*. At the same time, other species thrive in the less acidic environment. “There are two microbiomes of the stomach, one with *H. pylori* and one without,” says Timothy Wang.

Perhaps, he says, *H. pylori* should be reconceived as a commensal—basically neutral, providing that it stays where it belongs and remains in balance with the other species in its web of life, host included. For evidence, he turns to ancient history. Stomach ulcers are a 20th century affliction, but DNA evidence suggests that *H. pylori* has been present in the human gut for more than 200,000 years—long before our population outgrew the cradle of civilization. “In the vast majority of people—85 to 90 percent of people—they experience no harm from the bacteria and there may be some protective effects,” he says. “Maybe in the past, we never survived long enough to get ulcers.”

The same might be said of gastric cancer, another hazard of long-term exposure to *H. pylori*. Among people whose gut hosts the bacterium, between 1 percent and 2 percent develop malignancies in

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the lining of their stomachs. Early in his career, Timothy Wang leveraged that statistic to develop the first rodent model of gastric cancer, by exposing mice to *Helicobacter felis*, a close relative of *H. pylori*. Since then, he has devoted much of his research portfolio to uncovering the mechanisms by which the bacterium induces malignancy. “It turned out, in our animal models—and we found this was also true in human patients—that when the cancer actually develops it’s in the setting of severe inflammation,” he says. “It was our hypothesis that the bacteria were modulating the host immune response leading to the cancer. This is the view of many people in the microbiome field—that

it's through the modulation of inflammation that a lot of the effects occur."

In particular, Timothy Wang and his collaborators have homed in on a curious detail: While the presence of *H. pylori* is predictive of future cancer risk, the bacterium seems to disappear around the same time malignancies actually take root. In a series of experiments with knockout mice in a germ-free facility, Timothy Wang and collaborators have shown a role for the additional bacte-

“Right now a lot of microbiome research is correlative. Actually showing causality is difficult.”

ria whose populations thrive as *H. pylori* pushes the stomach pH ever higher. “We demonstrated unequivocally that late-stage progression of gastric cancer is inhibited in a germ-free facility,” says Dr. Wang. “It’s really the bacterial overgrowth that is responsible for the progression of gastric cancer.”

Stomach acidity is not the only host environmental condition that constrains *H. pylori*. It turns out that modern living itself—our penchant for antibiotics and a class of antacids known as proton pump inhibitors—has precipitated a dramatic decrease in the number of people whose gut microbiome even contains *H. pylori*. Meanwhile, esophageal cancer seems to have reached epidemic proportions in developed countries. The divergent trends have led some scientists to speculate that perhaps *H. pylori* actually protects against esophageal cancer.

To test the association, Timothy Wang has partnered with Julian Abrams, MD, an expert in esophageal cancer. “We think that the upper GI tract microbiome plays a big role in the development of gastric cancer,” says Dr. Wang. “We’re starting to see that at the junction of the esophagus and stomach, the microbiome probably changes based on whether or not *H. pylori* is present in the stomach.” To reveal the mechanisms by which the bacterium exerts its influence, Dr. Wang is running a new series of experiments in a germ-free facility, this time with mice susceptible to esophageal cancer. “Perhaps *H. pylori* alters the stomach microbiome, thereby altering the esophageal microbiome, and that somehow affects the

development of esophageal adenocarcinoma,” he says. “At least, that’s the hypothesis.”

Another possible explanation for the negative association of *H. pylori* with esophageal cancer is that *H. pylori* is our microbial canary in the coal mine—just one among many gut microbes whose populations are failing under the onslaught of antibiotics and the rise of modern living. “There are thousands of species and there’s a dynamic balance,” says Dr. Lipkin. “We know that bacteriophages control levels of bacterial populations, but we don’t yet know the details.” The hazard is a slow, steady constriction of the microbial populations that hold our immune system in balance—and potentially the rise of far more vicious pathogens. As Dr. Lipkin notes, “Nature abhors a vacuum.” Perhaps, says Timothy Wang, *H. pylori* actually has nothing to do with esophageal cancer and some other microbe—not yet detected or named—is the real protector of esophageal health. “It’s like frogs disappearing in the swamps,” he says. “We’re not really that concerned about the frogs; it’s what the frogs represent, which is the disappearance of a lot of other species.”

Exploring Mechanisms of Inflammation

A growing body of research—including work by Timothy Wang—delves into the mechanisms by which inflammation mediates the microbial ecology and its association with malignancy. “When you get inflammation, even if that inflammation is not due to the bacteria, it changes the bacteria around that tissue,” says Dr. Wang. “It’s dysbiosis—abnormal bacterial colonization.” While the association is robust, scientists have struggled to demonstrate how A leads to B leads to C—or whether, perhaps, C initiates A. “The chicken or the egg is complicated. Does the inflammation come first or the change in the bacteria? Do they occur simultaneously? It’s hard to say. Right now a lot of microbiome research is correlative. Actually showing causality is difficult.”

Hepatologist Elizabeth C. Verna, MD, has turned her attention to the mechanisms by which the ecology of the digestive tract affects inflammation of the liver, that rubbery, three-pound organ tucked under the rib cage to filter and detoxify our blood. “The gut in a normal person is filled with millions of microbes, but for the most part the intestine creates a barrier to contain those microbes,” she explains. “And that barrier is tightly regulated.” Chronic liver disease, including

hepatitis C, the focus of Dr. Verna’s prior research, turns the gut barrier into a leaky sieve. It also triggers shifts in gut ecology to favor more pathogenic organisms, which can throw blood chemistry for a loop. The combination puts a dual strain on the liver, tasked with cleaning every ounce of blood that filters from the digestive tract before it moves on through the rest of the body. Says Dr. Verna: “Bacterial particles that leak across the gut and changes in metabolism both have a very strong impact on inflammation and scarring in the liver.”

As a clinician with Columbia’s Center for Liver Disease and Transplantation, Dr. Verna sees the aftermath of inflammation and scarring, the hallmarks of chronic liver disease. Each year, she and her colleagues manage postoperative care for 140 patients recuperating from a liver transplant, a major abdominal surgery followed by a lifelong protocol of immune-suppressing medications. In December, the National Institute of Diabetes and Digestive and Kidney Diseases awarded her a \$607,941 grant for a four-year project to investigate the role of the intestinal microbiome in recurrent disease following liver transplantation. “I’m interested in mechanisms of liver injury and scarring in general and in particular among liver transplant patients,” she says, “because they



Anne-Catrin Uhlemann, MD, PhD

have an abnormal intestinal microbiome at the time of transplant, which puts them at high risk of having this mechanism of liver injury. Transplant recipients can have very severe outcomes, including mortality.”

Over the past three years, Dr. Verna has enrolled individuals willing to provide serial blood and stool samples as they prepare to undergo a liver transplant and in the months and years following the procedure. To track participants’ digestive microbes, Dr. Verna and her collaborators, including bacteriologist and infectious diseases specialist Anne-Catrin Uhlemann, MD, PhD, perform 16S rRNA sequencing of the stool samples. To approximate the burden of digestive microbes within the blood, the scientists have homed in on endotoxins, bacterial cell wall components the liver has failed to filter, and immune markers associated with intestinal permeability. Over time, they plan to collect additional samples from the digestive tract to expand their data set. It is a particularly exciting project, says Dr. Verna. “This work is at the interface between big data—large bodies of information about large numbers of people, each of whom has a large population of digestive microbes—and the specificity of personalized medicine to assess an individual patient’s risk and how we might modulate that risk.”

Such efforts got a technical boost this year when the Department of Medicine committed funds to launch a Microbiome Core Facility for researchers across campus. Services include guidance on various elements of study design; protocols for collecting, storing, and shipping sensitive samples for genetic sequencing; and analyses. In addition, the facility provides assistance with DNA extraction and batch samples from multiple labs to make sequencing more cost-effective across the institution. Dr. Uhlemann leads the facility. “Our idea,” says Dr. Uhlemann, “is to enable researchers without a large experimental lab or computational expertise to do large-scale, data-intensive studies.”

Preliminary analyses of Dr. Verna’s liver transplant data have revealed a critical insight: Timing matters. “Most patients, to some degree, experience this process of bacterial translocation early after the transplant,” she says. The observation comes as no surprise, given the dysbiosis associated with major abdominal surgery and the drugs used to suppress organ rejection. The data point most relevant for long-term prognosis, then, is how long it takes for a patient’s gut to



Elizabeth C. Verna, MD

recalibrate. “Those who seem to develop disease are those who still have the biomarkers of translocation three months after surgery.”

Of particular importance to the scientific community, says Dr. Verna, are the implications of that preliminary analysis for study design: At least in the case of recovery from a liver transplant, it’s not enough to take a single snapshot of the digestive microbiome. “The key to studying this, which is really missing in the literature, is that you have to study patients over time. If you just have a cross-section of one patient at one moment in time, you’re not really going to understand what’s happening.”

The next phase of analysis will focus on predictive features of the microbial census, as well as correlations with such clinical details as the rate of metabolism of various drugs within the anti-suppression protocol and the incidence of metabolic disruption among transplant survivors. “The idea is that eventually we will know enough to target specific microbes or specific metabolic pathways,” says Dr. Verna, “so we can fine-tune things to maximize each patient’s post-transplant outcomes.”

Understanding Why Microbes Go Bad

While Dr. Verna investigates a single clinical outcome—triggers for scarring and inflammation within the liver—Dr. Han has homed in on how a single bacterium can toggle from commensal to pathogen. In the case of *F. nucleatum*, such host

factors as diet, immune status, smoking, and hydration all influence just how much weight the microbe can throw around the ecological playground. Blood chemistry, too—in the form of hormones and blood sugar levels—influences which microbes live or die. Pregnancy, it turns out, is a double whammy for the oral cavity.

Women and their physicians have long known that intense hormonal shifts—puberty, pregnancy, menopause—seem to increase susceptibility to the swollen, bleeding gums and other symptoms of periodontal disease caused by rampant microbial growth in the oral cavity. During pregnancy, the immune suppression that prevents a woman’s body from rejecting her fetus seems to exacerbate the situation. Pregnancy-related gingivitis afflicts approximately 50 percent to 75 percent of pregnant women. “It’s an often overlooked condition because it’s self-limiting,” says Dr. Han. “After childbirth, hormonal levels are restored, and the condition subsides.”

Among healthy individuals with regular access to dental care, *F. nucleatum* is no big deal and it is rarely found beyond the oral cavity. Among those with compromised immune function—someone fighting off a respiratory infection or some other illness—doctors often detect the nonmotile anaerobe far from where it belongs, including within the joints, lungs, and reproductive tract. In an August 2015 review for *Current Opinion in Microbiology*, Dr. Han recorded the microbe’s

association with appendicitis, atherosclerosis, and cerebral aneurysm, among others. “This bacterium has been implicated in a wide array of diseases,” says Dr. Han. “It’s been associated with cardiovascular disease and isolated from abscesses in every organ, even the brain.”

Just how, exactly, does a nonmotile, oral microbe make its way into the joints, across the blood-brain barrier, and throughout the reproductive tract? So far, scientists have identified two modes by which *F. nucleatum* makes its journey: through our digestive

tract and in our blood. “We have tons of bacteria in our saliva,” says Dr. Han. “It’s like a washing solution.” And ultimately, the bacteria that mix with our food as we chew and swallow make their way to the stomach. “Whether they proceed to the intestine depends on how resistant or sensitive they are to stomach acid. It’s a numbers game.” Bacteria in the *Shigella* genus, for example, are highly acid-tolerant; exposure to just 20 of the rod-shaped anaerobes can cause a severe case of dysentery. The comma-shaped *Vibrio cholerae*, on the other hand, is extremely acid-sensitive and a very high dose of the shellfish-borne bacterium is required to cause symptoms. “That passage doesn’t need a lot of convincing,” says Dr. Han. “People understand that you swallow these microbes all the time, but your exposure depends on how heavy your oral inoculum is.”

To explain the presence of *F. nucleatum* in meningitis and the joints of people with rheumatoid arthritis, as well as amniotic fluid, placental tissue, and chorioamniotic membranes of women who experienced premature labor, Dr. Han has focused her investigations on blood-borne pathways. “For the bacteria to spread to so many parts, the only logical explanation is the circulation,” she says. “It’s the only passage that can reach every part of the body.”

Like a mosquito inadvertently introducing Zika or West Nile virus as it sucks down its dinner, a nick or cut in the mouth—whether from vigorous flossing or chomping on a piece of toast—allows bacteria within the oral cavity to pass into the bloodstream. As with the symptoms of *H. pylori* infection, host susceptibility factors into the gingivitis equation, along with the unique census of an individual’s oral microbiome. “Each person’s susceptibility to dental bacteremia is different,”

says Dr. Han. “For some people, once you have the bleeding, once the bacteria enter circulation, they survive only a few minutes; for others, the bacteria persist for hours.”

In 2004, the Journal of Infection and Immunity published a paper describing the first animal model elucidating the role of *F. nucleatum* in adverse pregnancy outcomes. By injecting the bacterium into the tail vein of pregnant mice, Dr. Han and colleagues mimicked dental bacteremia, demonstrating how *F. nucleatum* invades placental tissue and, ultimately, the amniotic fluid, causing preterm birth and miscarriage without causing systemic maternal infections. In 2007, Dr. Han’s paper in the Journal of Immunology revealed the biochemical mechanism at play: *F. nucleatum* stimulates placental inflammation by hijacking receptors in the immune system; treatment with an anti-inflammatory agent reduced the risk of fetal death.

Two years later, a woman who had been afflicted by excessive gum bleeding during an otherwise uncomplicated pregnancy approached Dr. Han after having a miscarriage just two weeks before her due date. Dr. Han’s team dug into the case, confirming that identical strains of *F. nucleatum* isolated during autopsy from the fetus’s lungs and stomach were also present in the woman’s mouth, but not in any of the other samples they collected from her. Almost certainly, they concluded, the woman had suffered pregnancy-related gingivitis; it was only when a brief respiratory illness further weakened her immune system that the oral commensal persisted long enough in her bloodstream to invade the placenta, infuse the amniotic fluid, and infect the fetus. The Journal of Obstetrics and Gynecology published the full report in 2010.

While vaginal microbes have frequently been implicated in miscarriage, preterm labor, and other adverse pregnancy outcomes, microbial commensals associated with other parts of the human habitat—like *F. nucleatum*—may be implicated in many of the adverse outcomes doctors previously dismissed as “idiopathic.” Perhaps, Dr. Han and co-authors speculated, the failure to identify *F. nucleatum* as the cause owes to the technical difficulty of microbial detection using conventional culturing techniques, especially for anaerobic species. In the Obstetrics and Gynecology case study, Dr. Han and colleagues deployed a genomic approach, using polymerase chain reactions to amplify genes within the samples they had collected, then checked them against the Human Microbiome database.

“New molecular techniques have made it possible to identify organisms that could not be grown in culture. That’s been an enormous boon.”

Who’s Who

- Julian Abrams, MD, assistant professor of medicine and epidemiology
- Sankar Ghosh, PhD, the Silverstein and Hutt Family Professor of Microbiology & Immunology and chair, Department of Microbiology & Immunology
- Yiping W. Han, PhD, professor of microbial sciences (in dental medicine, microbiology & immunology, obstetrics & gynecology, and medical sciences in medicine) and a member of the Herbert Irving Comprehensive Cancer Center
- Ivaylo Ivanov, PhD, assistant professor of microbiology & immunology
- W. Ian Lipkin, MD, the John Snow Professor of Epidemiology, director of Columbia’s Center for Infection and Immunity, and professor of neurology and pathology
- Anne-Catrin Uhlemann, MD, PhD, Florence Irving Assistant Professor of Medicine
- Elizabeth C. Verna, MD, assistant professor of medicine and director of clinical research for the Transplant Initiative
- Harris Wang, PhD, assistant professor of systems biology (in pathology & cell biology)
- Timothy C. Wang, MD, the Dorothy L. and Daniel H. Silberberg Professor of Medicine, chief of the Division of Digestive and Liver Diseases in the Department of Medicine, and a member of the Herbert Irving Comprehensive Cancer Center



Timothy C. Wang, MD, and Yiping W. Han, PhD

PCR and other molecular assays have been vital to growth in the field, says Dr. Lipkin, whose team developed VirCapSeq-VERT, a technique that allows simultaneous testing for hundreds of different viruses and provides near complete sequences of their genomes. “New molecular techniques have made it possible to identify organisms that could not be grown in culture,” he says. “That’s been an enormous boon to identify organisms more rapidly, which has allowed us to think in terms of how we might more rapidly respond to acute infectious disease and implicate chronic and viral pathogens in cancer, even in those instances where we can’t grow them.”

Ever more affordable prices for PCR analysis are especially critical for boosting diagnostic accuracy in clinical settings, says Dr. Han. “Currently in the hospital laboratory, the gold standard is still the culturing method but we know that many bacteria that live in or on us are uncultivated or difficult to cultivate.” In the oral cavity, for example, scientists have identified as many as 700 species

(each of us hosts only a few hundred of those species). “Only half have been cultivated. The other half, we know they are there, but we don’t know what kind of growth conditions they require to grow in the laboratory setting.”

Scientists have their work cut out for them and Dr. Han has made the development of new microbial techniques a top priority for her research team. In April 2015, the *Journal of Clinical Microbiology* published Dr. Han’s description of a specialized mass spectrometry technique for identifying subspecies of *F. nucleatum*. In November 2015, Dr. Han and her collaborators received a patent for the polymerase chain reaction technique they used to detect bacterial pathogens in the Obstetrics and Gynecology case study. “One of the frontiers in microbiology is to cultivate the uncultivated,” she says. “In order to study pathogenic mechanisms, we need to know the conditions under which they thrive or die and we have to be able to cultivate them so we can manipulate and study them in the laboratory setting.”

Among the vital insights *F. nucleatum* has yielded to Dr. Han’s manipulations is its reliance on a unique adhesin protein the scientist dubbed FadA. “It’s one small molecule of only 129 amino acids,” she says, “but it can do a lot of things.” Key to *F. nucleatum*’s ability to colonize so many parts of the body is FadA’s connectivity with a type of receptor known as a cadherin, which serves as a cellular gatekeeper. “Cadherins are ubiquitous in our cells. You have vascular-endothelial, VE-cadherins, and epithelial, E-cadherins; neuron cells have N-cadherins, and in the placenta are P-cadherins.”

Like Spider-Man clinging to a wall, FadA can lock on to a wide array of sites by binding to the specialized cadherins it encounters throughout our bodies. “As a microbiologist, I’m amazed by how smart and efficient these bugs are,” says Dr. Han. “They have such a tiny genome—only 2 million base pairs. However, they can manipulate us, their hosts, so efficiently, making us work for them.”

FadA goes far beyond merely clinging to the cadherins; it leverages something of a Trojan horse effect. “Once FadA binds to cadherin, it loosens the tight junction, like a key,” Dr. Han explains. “Not only can *Fusobacterium* get into the circulation by itself, but whatever bacteria are in the vicinity can come along. It’s like a facilitator, an enabler.”

In their investigation of the role of *Fusobacterium nucleatum* in colorectal cancer, Timothy Wang and Dr. Han are drawing on her investigations of the particularly diabolical synergy of FadA with E-cadherin, a well-known cancer suppressor, as well as his expertise in *H. pylori*, particularly its knack for altering its environment. Already, they have demonstrated that in knockout mice engineered with a predisposition for colon polyps, *F. nucleatum* can accelerate activation of the cancer. “It’s proof of principle that the bacteria alone are not sufficient to cause colon cancer,” says Dr. Wang, “but can wake up dormant cancer stem cells to go on to become tumors in the large intestine.”

The prospect of showing that a single bacterium can induce cancer is an exciting one, says Dr. Wang, but the work will not be that simple. “In most cases, it’s not going to be a single bacterium,” says Dr. Wang, noting that he and Dr. Han have a lot of genomic sequencing and bioinformatics work in their future. “These things live in student dorms with a lot of their friends. They’re like fraternities, in a way. Just looking at one at a time makes it really difficult to figure out the source of the noise late at night.” ♦