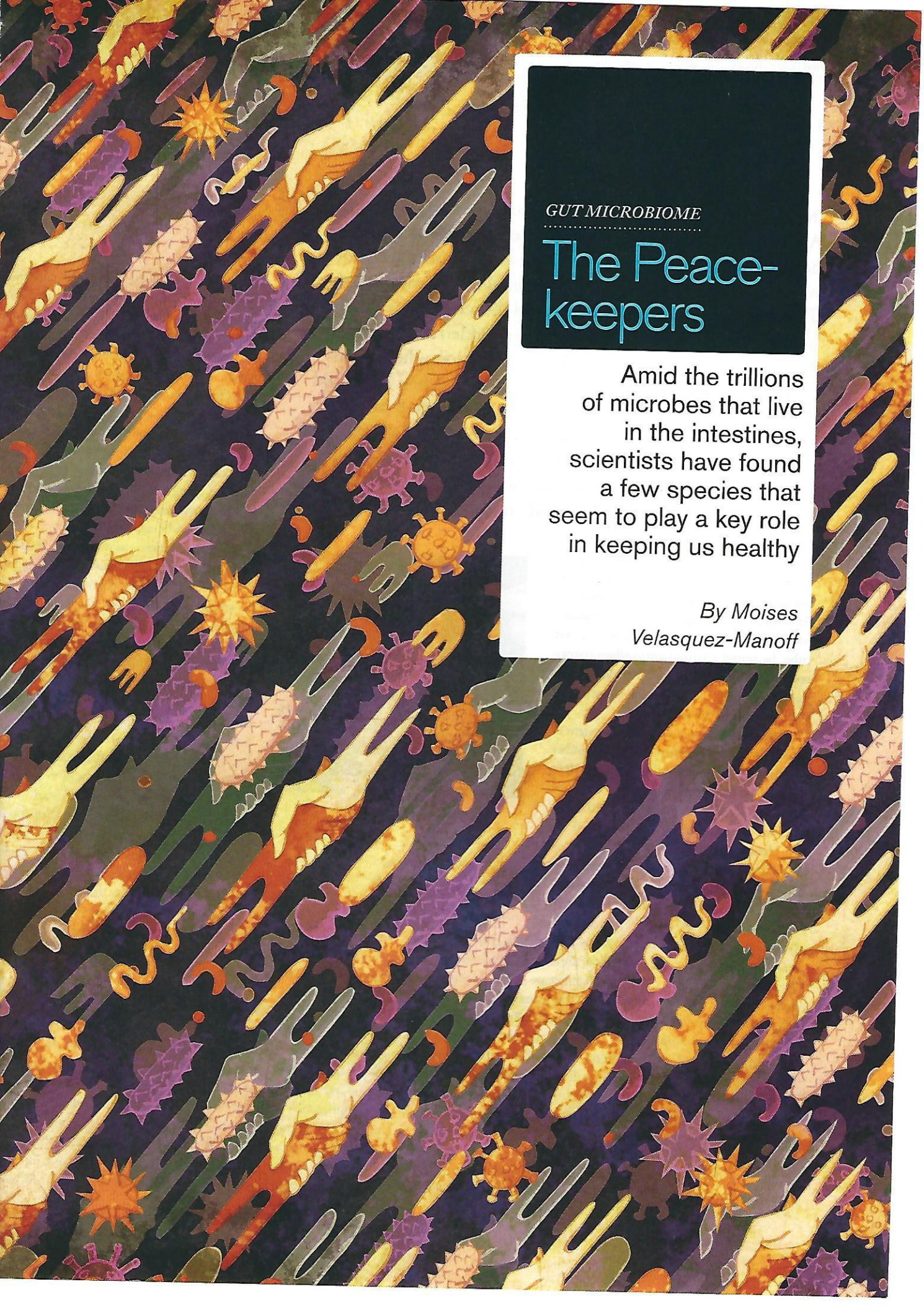


GUT MICROBIOME

The Peace-keepers

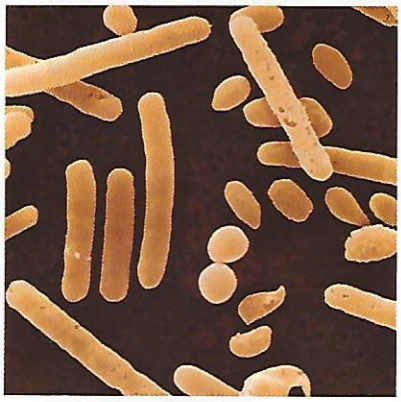
Amid the trillions of microbes that live in the intestines, scientists have found a few species that seem to play a key role in keeping us healthy

By Moises Velasquez-Manoff

IN THE MID-2000s Harry Sokol, a gastroenterologist at Saint Antoine Hospital in Paris, was surprised by what he found when he ran some laboratory tests on tissue samples from his patients with Crohn's disease, a chronic inflammatory disorder of the gut. The exact cause of inflammatory bowel disease remains a mystery. Some have argued that it results from a hidden infection; others suspect a proliferation of certain bacteria among the trillions of microbes that inhabit the human gut. But when Sokol did a comparative DNA analysis of diseased sections of intestine surgically removed from the patients, he observed a relative depletion of just one common bacterium, *Faecalibacterium prausnitzii.* Rather than "bad" microbes prompting disease, he wondered, could a single "good" microbe prevent disease?

Sokol transferred the bacterium to mice and found it protected them against experimentally induced intestinal inflammation. And when he subsequently mixed *F. prausnitzii* with human immune cells in a test tube, he noted a strong anti-inflammatory response. Sokol seemed to have identified a powerfully anti-inflammatory member of the human microbiota.

Each of us harbors a teeming ecosystem of microbes that outnumbers the total number of cells in the human body by a factor of 10 to one and whose collective genome is at least 150 times larger than our own. In 2012 the National Institutes of Health completed the first phase of the Human Microbiome Project, a multimillion-dollar effort to catalogue and understand the microbes that inhabit our bodies. The microbiome varies dramatically from one individual to the next and can change quickly over time in a single individual. The great majority of the microbes live in the gut, particularly the large intestine, which serves as an anaerobic digestion chamber. Scientists are still in the early stages of exploring the gut microbiome, but a burgeoning body of research suggests that the makeup of this complex microbial ecosystem is closely linked with our immune function. Some researchers now suspect that, aside from protecting us from infection, one of the immune system's jobs is to cultivate, or "farm," the friendly microbes that we rely on to keep us healthy. This "farming" goes both ways, though. Our resident microbes seem to control aspects of our immune function in a way that suggests they are farming us, too.



UNRULY NAMESAKE: Clostridium difficile, *a bacterial scourge in hospitals, is a distant relative of benign "clostridial cluster" microbes that seem to play a key role in gut health.*

Independent researchers around the world have identified a select group of microbes that seem important for gut health and a balanced immune system. They belong to several clustered branches of the clostridial group. Dubbed "clostridial clusters," these microbes are distantly related to *Clostridium difficile,* a scourge of hospitals and an all too frequent cause of death by diarrhea. But where *C. difficile* prompts endless inflammation, bleeding and potentially catastrophic loss of fluids, the clostridial clusters do just the opposite—they keep the gut barrier tight and healthy, and they soothe the immune system. Scientists are now exploring whether these microbes can be used to treat a bevy of the autoimmune, allergic and inflammatory disorders that have increased in recent decades, including Crohn's and maybe even obesity.

F. prausnitzii was one of the first clostridial microbes to be identified. In Sokol's patients those with higher counts of *F. prausnitzii* consistently fared best six months after surgery. After he published his initial findings in 2008, scientists in India and Japan also found *F. prausnitzii* to be depleted in patients with inflammatory bowel disease. Sokol was particularly intrigued by the results from Japan. In East Asian populations the gene variants associated with inflammatory bowel disease differ from the gene variants in European populations. Yet the same bacterial species—*F. prausnitzii*—was reduced in the guts of those in whom the disease developed. This suggested that whereas different genetic vulnerabilities might underlie the disorder, the path to disease was similar: a loss of anti-inflammatory microbes from the gut. And although Sokol suspects that other good bacteria besides *F. prausnitzii* exist, this similarity hinted at a potential one-size-fits-all remedy for Crohn's and possibly other inflammatory disorders: restoration of peacekeeping microbes.

MICROBIAL ECOSYSTEMS

ONE OF THE QUESTIONS central to microbiome research is why people in modern society, who are relatively free of infectious diseases, a major cause of inflammation, are so prone to inflammatory, autoimmune and allergic diseases. Many now suspect that society-wide shifts in our microbial communities have contributed to our seemingly hyperreactive immune systems. Drivers of these changes might include antibiotics; sanitary practices that are aimed at limiting infectious disease but that also hinder the transmission of symbiotic microbes; and, of course, our high-sugar, high-fat modern diet. Our microbes

Why Microbiome Treatments Could Pay Off Soon

Effective interventions may come before all the research is in *By Rob Knight*

Today we are at an exciting threshold of biology. Advances in DNA sequencing, coupled with high-end computation, are opening a frontier in new knowledge. Obtaining genetic information and obtaining insight from it have never been cheaper. The potential for curing previously incurable diseases, including chronic ones, seems immense. If this sounds familiar, you might be thinking that you heard it 15 years ago, when the Human Genome Project was in full swing. Many feel that genomic medicine has not yet delivered on its promise. So what is different this time with the microbiome? For one thing, you cannot really change your genome, but each of us has changed our microbiome profoundly throughout our lives. We have the potential not just to read out our microbiome and look at predispositions but to change it for the better.

What is most exciting at this stage is that we have mouse models that let us establish whether changes in the microbiome are causes or effects of disease. For example, we showed in collaborative work with Jeffrey I. Gordon's laboratory at Washington University in St. Louis last year that transferring the microbes from an obese person into mice raised in a bubble with no microbes of their own resulted in fatter mice. Normally, germ-free mice exposed to a mouse with microbial-based obesity would themselves become obese, but we could design a microbial community taken from lean people that protected against this weight gain. Similarly, we could take microbes from Malawian children with kwashiorkor, a profound nutritional deficiency, transplant them into germ-free mice and transfer the malnutrition, although the mice that received the microbes from the healthy identical twins of the sick children did fine. Remarkably, the mice that got the kwashiorkor microbiome, which lost 30 percent of their body weight in three weeks and died if untreated, recovered when given the same peanut butter–based supplement that is used to treat children in the clinic.

We have the potential not just to read out our microbiome and look at predispositions but to change it for the better.

The germ-free mice are far too expensive to deploy in Malawi, Bangladesh and the other sites in the Mal-ED (pronounced "mal-a-dee") global network for the study of malnutrition and enteric diseases collaboration with which we work. Thus, we are trying to move from the mouse model to a test-tube model and ultimately to a primarily computational model based on DNA sequencing that is so inexpensive, it is effectively free.

With crowdfunded projects such as American Gut, which already has thousands of participants who have had their microbiomes sequenced, and studies of people whose lives are very different from modern Western civilization, such as the Hadza of Tanzania, Yanomami of Venezuela and Matsés of Peru, we may be able to replenish our ancestral microbes and discover new ones that help to maintain health for individuals or entire populations. A good analogy is iodizing salt: Instead of understanding in detail why some people but not others were susceptible to cretinism and goiter, adding a nutrient to the food supply greatly reduced incidences of these diseases. Perhaps the same type of intervention is possible using some of the microbes that we are now discovering Westerners lack.

Rob Knight *is a computational biology pioneer, co-founder of the American Gut Project and director of the new Microbiome Initiative at the University of California, San Diego.*

Illustrations by Gluekit

eat what we eat, after all. Moreover, our particular surroundings may seed us with unique microbes, "localizing" our microbiota.

The tremendous microbial variation now evident among people has forced scientists to rethink how these communities work. Whereas a few years ago they imagined a core set of human-adapted microbes common to us all, they are now more likely to discuss core functions—specific jobs fulfilled by any number of microbes.

Faced with the many instances of a misbehaving immune system, it is tempting to imagine that rather than having developed a greater vulnerability to many diseases, we actually suffer from just one problem: a hyperreactive immune system. Maybe that tendency has been enabled, in part, by a decline or loss of key anti-inflammatory microbes and a weakening of their peacekeeping function.

Antibiotics may deplete the bacteria that favorably calibrate the immune system, leaving it prone to overreaction.

In ecosystem science, "keystone species" have an outsize role in shaping the greater ecosystem. Elephants, for example, help to maintain the African savanna by knocking down trees, thus benefiting all grazing animals. The concept may not apply perfectly to our inner microbial ecosystems—keystone species tend to be few in number, whereas peacekeeping microbes such as *F. prausnitzii* are quite numerous. Yet it provides a useful framework to think about those clostridial microbes.

They seem to occupy a particular ecological niche, sidled right up against the gut lining, which allows them to interface more closely with us, their hosts, than other members of the gut microbiota. They often specialize in fermenting dietary fiber that we cannot digest and produce by-products, or metabolites, that appear to be important for gut health. Some of the cells that line our colon derive nourishment directly from these metabolites, not from the bloodstream. And when no fiber comes down the hatch, the clostridial microbes and others can switch to sugars in the intestinal mucous layer—sugars we produce, apparently, to keep them happy. In fact, they seem to stimulate mucus production.

Kenya Honda, a microbiologist at Keio University in Tokyo, was among the first to uncover the critical role of clostridial microbes in maintaining a balanced immune system. To study how native microbes affect animals, scientists decades ago developed the germ-free mouse: an animal without any microbiota whatsoever. These rodents, delivered by cesarean section and raised in sterile plastic bubbles, can exist only in labs. Of the many oddities they present—including shrunken heart and lungs and abnormalities in the large intestine—Honda was particularly intrigued by their lack of cells that prevented immune overreaction, called regulatory T cells, or Tregs. Without these cells, the mice were unusually prone to inflammatory disease.

Honda wanted to know which of the many intestinal species might induce these suppressor cells. Soon after Sokol identified the anti-inflammatory effects of *F. prausnitzii,* Honda began whittling away at the gut microbiota of mice by treating them with narrow-spectrum antibiotics. The animals' Tregs declined after a course of vancomycin. With their ability to restrain their immune reaction hobbled, the mice became highly susceptible to colitis, the rodent version of inflammatory bowel disease and allergic diarrhea. Honda found he could restore the Tregs and immune equilibrium of the mice just by reinstating 46 native clostridial strains.

Honda repeated the exercise with human-adapted microbes obtained from a healthy lab member. He extracted just 17 clostridial species this time that, in mice, could induce a full repertoire of Tregs and prevent inflammation. These human-adapted microbes specialized in nudging the immune system away from inflammatory disease. They came from branches of the clostridial group labeled clusters IV, XIVa and XVIII. *F. prausnitzii* belongs to cluster IV.

Vedanta Biosciences recently formed to try to turn Honda's 17-strain "clostridial cocktail" into a treatment for inflammatory disease. If the company's efforts are successful, it could signal the arrival of the next generation of probiotics—human-adapted microbes to treat immune-mediated disease—and all derived from one member of Honda's lab. As always, it is unclear if what works in lab mice will translate to humans. Sokol has his doubts. He recently identified a type of regulatory T cell that is unique to humans and that is deficient in people with inflammatory bowel disease. He questions if Honda's cocktail, which has been developed in mice, will activate these cells in people.

TROUBLE WITH ANTIBIOTICS

EVEN IF THE COCKTAIL falls short, Honda's meticulous demonstration of a link between antibiotics and vulnerability to inflammatory disease has raised a troubling question. A number of studies have found a small but significant correlation between the early-life use of antibiotics and the later development of inflammatory disorders, including asthma, inflammatory bowel disease and, more recently, colorectal cancer and childhood obesity. One explanation for this association might be that sickly people take more antibiotics. Antibiotics are not the cause, in other words, but the result of preexisting ill health.

Honda's studies suggest another explanation: antibiotics may deplete the very bacteria that favorably calibrate the immune system, leaving it prone to overreaction. Brett Finlay, a microbiologist at the University of British Columbia, has explored this possibility explicitly. Early-life vancomycin treatment of mice increased the animals' risk of asthma later, he found, in part by depleting those very same clostridial bacteria identified by Honda. The corresponding population of suppressor cells collapsed. And the animals became less able to restrain their immune responses when encountering allergens later.

These dynamics may also apply to other diseases. Earlier this year Cathryn Nagler, an immunologist at the University of Chica-

The Gene–Microbe Link

Evidence that genes shape the microbiome may point to new treatments for common diseases *By Ruth E. Ley*

The ecology of the gut microbiome may trigger or contribute to a variety of diseases, including autoimmune disorders and obesity, research suggests. Factors such as early environment, diet and antibiotic exposure have a lot to do with why people differ from one another in the composition of their microbiomes. But specific gene variants are also linked to greater risks of developing many of these diseases. Do your genes act on your microbiome, which in turn promotes disease?

One way researchers have addressed this question is to pick specific genes that are good candidates—for instance, those with a strong link to a disease that also has a microbiome link—and examine whether people who carry mutations that are known to increase the risk of a certain disease also have microbiomes that differ from those who do not have the mutations. A team led by Dan Frank at the University of Colorado Denver took this approach and revealed that specific variants of the *NOD2* gene that confer a high risk of developing inflammatory bowel disease to their carriers are also associated with an altered intestinal microbiome.

A powerful and broader way to look for an effect of human genetic variation on the microbiome is to compare twins. Identical twins share nearly 100 percent of their genes; fraternal twins, 50 percent. Co-twins are raised together, so the environmental effects on their microbiomes should be about the same. If the microbiomes of the identical twins are more alike within a twinship than those of the fraternal twins, we can conclude that genes have played a role. If variation within twinships of each kind is about the same, we can say a shared genome has had no additional effect.

A powerful way to look for an effect of human genetic variation on the microbiome is to compare twins.

Early twins studies were based on fewer than 50 twin pairs and could not detect any greater similarities in the microbiomes of identical twins compared with those of fraternal twins. But recent work my laboratory at Cornell University conducted with researchers at King's College London compared nearly 500 twin pairs, a sample size sufficient to show a marked genetic effect on the relative abundance of a specific set of gut microbes. Furthermore, so-called heritable microbes—the bacteria most influenced by host genetics—were more abundant in lean twins than obese ones.

Experiments in germ-free mice showed that one gut bacterium in particular, *Christensenella minuta,* can influence the phenotype—the composite of observable characteristics or traits—of the host. Germ-free mice live in sterile bubbles—and they are very skinny. When they are given a microbiome in the form of a fecal transplant from a human donor, however, they plump up within a day or two because the bacteria help them digest their food and develop a proper metabolism. We found that if *C. minuta* was added to the feces of an obese human donor, the recipient mice were thinner than when *C. minuta* was not added. Results showing *C. minuta* has an effect of controlling fat gain in the mouse match data that reveal lean people have a greater abundance of *C. minuta* in their gut than obese people.

This is evidence that a person's genes can influence the gut microbiome's composition and in turn can shape the individual's phenotype. Further work will show what specific genes are involved as well as how the microbiome may be reshaped to reduce risk of developing chronic inflammatory diseases within the context of a person's genotype, suggesting potential new approaches to treating obesity-related diseases.

Ruth E. Ley *is an associate professor of molecular biology and genetics at Cornell University.*

go, knocked out the clostridial bacteria with antibiotics and then fed the animals peanut protein. Without those microbes and their corresponding Tregs present, the protein leaked through the gut barrier into circulation, prompting the rodent version of a food allergy. She could prevent the sensitization just by introducing those clostridial bacteria.

One key difference between mice with and without the clostridial clusters was how many mucus-secreting cells they possessed. Animals that harbored the clostridial clusters had more. That may have far-reaching consequences. Mucus, scientists are finding, contains compounds that repel certain microbes, maintaining a tiny distance between them and us. But it also carries food for other bacteria—complex, fermentable sugars that resemble those found in breast milk. Lora Hooper, a microbiologist at the University of Texas Southwestern Medical Center in Dallas, calls this dual function the "carrot" and the "stick." Mucus serves both as an antimicrobial repellent and a growth medium for friendly bacteria.

This phenomenon matters for several reasons. As Nagler's experiments suggest, one way these clostridial clusters may promote gut health and a balanced immune system is by ensuring a healthy flow of mucus. Just as those elephants help to maintain the African savanna, these microbes may favorably shape the greater gut ecosystem by stimulating secretion of the sugars other friendly microbes graze on.

Conversely, scientists observe defects in the mucous layer in other disorders, particularly inflammatory bowel disease, where these clostridial bacteria are often depleted. The question has always been which comes first: defects in mucus secretion and the selection of an aberrant community of microbes or acquisition of an aberrant community of microbes that thins the mucous layer and increases vulnerability to disease? Both factors may work together.

In 2011 scientists at the University of Colorado Boulder sampled people with variants of a gene called *NOD2* associated with inflammatory bowel disease. No one quite understands how these variants of the gene, which codes for a microbial sensor, increase the risk of disease. Study participants included people both with and without disease. Those suffering from inflammatory bowel disease had reduced counts of clostridial bacteria, the scientists found. But more surprising, people who did not have disease but who carried the predisposing *NOD2* variants also had a relative depletion of clostridial clusters. Their microbial communities seemed positioned closer to a diseaselike state.

The study seems to highlight the role of genes in determining the composition of gut microbiota and the vulnerability to Crohn's. But epidemiological surveys complicate the picture. A number of studies over the years have linked having fewer sanitary amenities in childhood with a lower risk of inflammatory bowel disease in adulthood. And a 2014 study from Aarhus University in Denmark found that among northern Europeans, growing up on a farm with livestock—another microbially enriched environment—halved the risk of being stricken with inflammatory bowel disease in adulthood.

These patterns suggest that perhaps by seeding the gut microbiota early in life or by direct modification of the immune system the environment can affect our risk of inflammatory bowel disease despite the genes we carry. And they raise the question of what proactive steps those of us who do not live on farms can take to increase our chances of harboring a healthy mix of microbes.

THE IMPORTANCE OF FIBER

ONE OF THE MORE SURPRISING discoveries in recent years is how much the gut microbiota of people living in North America differs from those of people living in rural conditions in Africa and South America. The microbial mix in North America is geared to digesting protein, simple sugars and fats, whereas the mix in rural African and Amazonian environments is far more diverse and geared to fermenting plant fiber. Some think that our hunter-gatherer ancestors harbored even greater microbial diversity in their guts. If we accept the gut microbiota of people in rural Africa and South America as proxies for those that prevailed before the industrial revolution, then, says Justin L. Sonnenburg, a microbiologist at Stanford University, the observed differences suggest North Americans and other Westernized populations have veered into evolutionarily novel territory.

What troubles Sonnenburg about this shift is that the bacteria that seem most anti-inflammatory—including the clostridial clusters—often specialize in fermenting soluble fiber. Fermentation produces various metabolites, including butyrate, acetate and propionate—some of the substances that produce underarm odor. Various rodent studies suggest that these metabolites, called short-chain fatty acids, can induce Tregs and calibrate immune function in ways that, over a lifetime, may prevent inflammatory disease. Fermentation by-products may be one way our gut microbes communicate with our bodies. One takeaway is to "feed your Tregs more fiber," as University of Oxford immunologist Fiona Powrie put it last year in the journal *Science*.

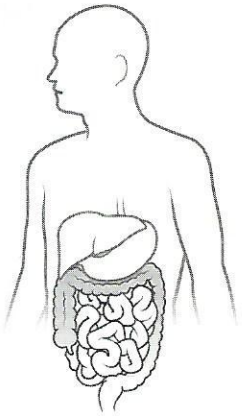
Yet the seeming importance of these metabolites has others puzzled. Many bacteria produce these short-chain fatty acids, and yet only a few microbes seem potently anti-inflammatory. So although production of these metabolites may be a prerequisite for microbes that favorably tweak the immune system, says Sarkis Mazmanian, a microbiologist at the California Institute of Technology, it is insufficient to explain why some bacteria are more anti-inflammatory than others. Other characteristics, such as how close they live to the gut lining or the molecules they use to prod the host immune system, must also play a role, he says.

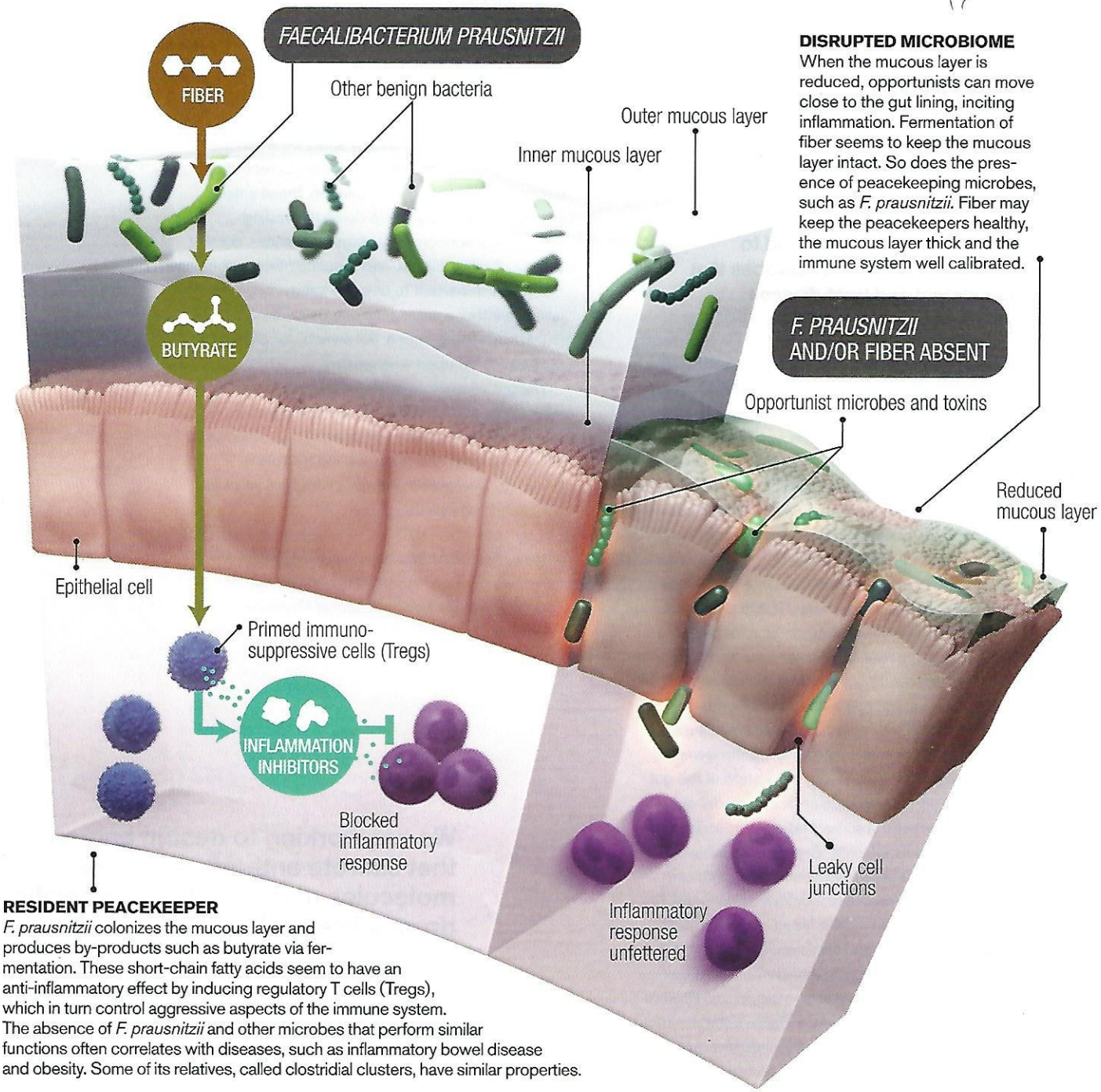
There is, however, an issue of sheer quantity. Some hunter-gatherers consumed up to 10 times as much soluble fiber as modern populations, and their bodies likely were flooded with far more fermentation by-products. Our fiber-poor modern diet may have weakened that signal, producing a state of "simmering hyperreactivity," Sonnenburg says, and predisposing us to the "plagues" of civilization. He calls this problem "starving our microbial self." We may not be adequately feeding some of the most important members of our microbiota.

Mouse experiments support the idea. Diets high in certain fats and sugars deplete anti-inflammatory bacteria, thin the mucous layer and foster systemic inflammation. Potentially dangerous opportunists bloom. In one intervention on human volunteers, University of California, San Francisco, microbiologist Peter Turnbaugh found that switching to a high-fat, high-protein diet spurred an expansion of bile-tolerant bacteria, one of which, *Bilophila wadsworthia,* has been

Your Microbes at Work: Fiber Fermenters Keep Us Healthy

The gut houses trillions of microbes. They eat what you eat. Many specialize in fermenting the soluble fiber in legumes, grains, fruits and vegetables. Certain microbial species are adept at colonizing the mucous layer of the gut. Mucus contains antimicrobial substances that keep the microbiota at a slight distance. But it also contains sugars such as those found in breast milk. Some microbes, often the same ones that specialize in fermenting fiber, can use these sugars as sustenance when other food is not available. The by-products of fiber fermentation nourish cells lining the colon. Some by-products pass into the circulation and may calibrate our immune system in a way that prevents inflammatory disorders such as asthma and Crohn's disease.





DISRUPTED MICROBIOME
When the mucous layer is reduced, opportunists can move close to the gut lining, inciting inflammation. Fermentation of fiber seems to keep the mucous layer intact. So does the presence of peacekeeping microbes, such as *F. prausnitzii.* Fiber may keep the peacekeepers healthy, the mucous layer thick and the immune system well calibrated.

RESIDENT PEACEKEEPER
F. prausnitzii colonizes the mucous layer and produces by-products such as butyrate via fermentation. These short-chain fatty acids seem to have an anti-inflammatory effect by inducing regulatory T cells (Tregs), which in turn control aggressive aspects of the immune system. The absence of *F. prausnitzii* and other microbes that perform similar functions often correlates with diseases, such as inflammatory bowel disease and obesity. Some of its relatives, called clostridial clusters, have similar properties.

SOURCE: "FEED YOUR TREGS MORE FIBER," BY JULIA BOLLRATH AND FIONA POWRIE, IN *SCIENCE*, VOL. 341; AUGUST 2, 2013

Illustration by AXS Biomedical Animation Studio

Microbiome Engineering

Synthetic biology may lead to the creation of smart microbes that can detect and treat disease

By Justin L. Sonnenburg

In the not too distant future each of us will be able to colonize our gut with genetically modified "smart" bacteria that detect and stamp out disease at the earliest possible moment. This scenario may sound like the premise for a sci-fi flick, but it is a very real possibility. Microbiome engineering holds great promise because of advances in the field of synthetic biology, which strives to create and rewire biological organisms so they perform desired tasks. Synthetic biologists are attempting to turn bacterial cells into the biological equivalent of the silicon wafer. These principles have been primarily applied to organisms for biofuel production, but the resulting techniques and genetic tool kit, when applied to our resident microbes, will have profound consequences for human health.

These resident microbes are adept at sensing what food is present, whether any pathogens are lurking and what the inflammatory state of the gut is—their survival depends on it. The model gut-resident bacterial species that we are using in our laboratory for initial tests, *Bacteroides thetaiotaomicron,* possesses more than 100 genetic circuits, each responsive to a different cue within the gut. If *B. thetaiotaomicron* "sees" pectin from an apple you recently ate, one circuit is triggered. If you eat a poached egg teeming with *Salmonella,* the resulting intestinal damage triggers a different circuit in *B. thetaiotaomicron.* Each of these circuits can be rewired so that the environmental cue elicits a designed response. Our early tests are focused on optimizing a DNA memory device for *B. thetaiotaomicron* so that we can record this bacterium's experiences as it transits through the gut. Invertible pieces of DNA are designed to flip, like switches, depending on what the bacterium detects. The two possible orientations (forward or flipped) of the DNA piece are akin to binary computer bits, which record a 1 or 0. Reading a genetic memory chip of a bacterium after it exits the gut will reveal fundamental principles about a single cell's journey through the digestive tract.

We are also working to design bacteria that secrete anti-inflammatory molecules when inflammation is detected, providing site-specific drug delivery within the intestine that automatically shuts off when the inflammation is eliminated. In addition to recording memories and treating inflammation, these smart bacteria may ultimately help combat invading pathogens, diagnose early stages of cancer, correct diarrhea or constipation, and regulate mood or behavior.

It is also important to develop safety mechanisms that ensure these organisms can be controlled. We are working to engineer a "kill switch" for eliminating engineered microbes if necessary.

The gut microbiota guides our immune system, metabolism, and even our moods and behavior. As we learn more about the specifics of our relationship with our resident microbes, we will be able to genetically manipulate them in a variety of ways to improve human health.

Justin L. Sonnenburg *is an assistant professor in the department of microbiology and immunology at the Stanford University School of Medicine.*

We are working to design bacteria that secrete anti-inflammatory molecules and provide site-specific drug delivery within the intestine.

linked to inflammatory bowel disease. On the other hand, preventing this skewing of the microbial self does not seem that difficult. In rodents, adding fermentable fiber to a diet otherwise high in fat keeps the "good" microbes happy, the mucous layer healthy and the gut barrier intact, and it prevents systemic inflammation. Taken together, these studies suggest that it is not only what is in your food that matters for your health but also what is missing.

The human studies are even more intriguing. Mounting evidence suggests that the systemic inflammation observed in obesity does not just result from the accumulation of fat but contributes to it. Scientists at Catholic University of Louvain in Belgium recently showed that adding inulin, a fermentable fiber, to the diet of obese women increased counts of *F. prausnitzii* and other clostridial bacteria and reduced that dangerous systemic inflammation. Weight loss was minor, but later analysis of this and two similar studies revealed that the intervention worked best on patients who, at the outset, already harbored clostridial clusters IV, IX and XIVa—some of the same clusters represented in Honda's cocktail. Those without the bacteria did not benefit, which suggests that once species disappear from the "microbial organ," the associated functions might also vanish. These individuals might not require ecosystem engineering so much as an ecosystem restoration.

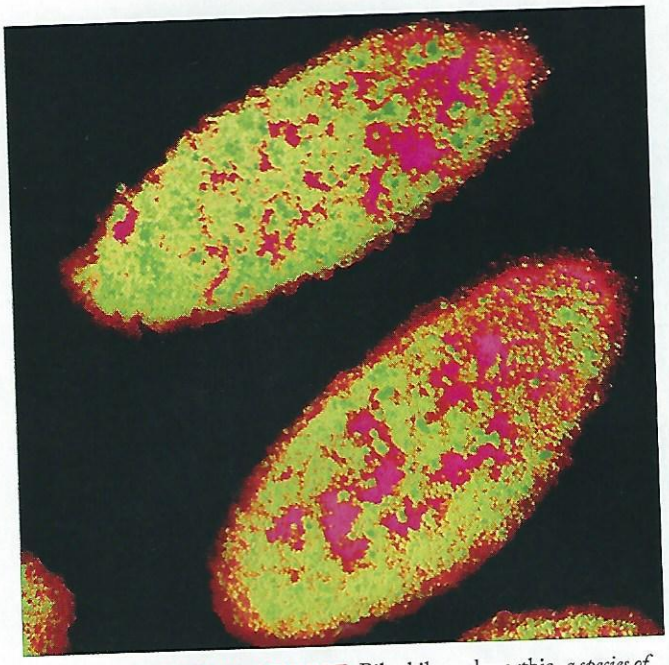
That possibility has also been tested. Several years ago Max Nieuwdorp, a gastroenterologist at the Academic Medical Center in Amsterdam, transplanted microbes from lean donors to patients recently diagnosed with metabolic syndrome, a cluster of symptoms that often predicts type 2 diabetes. The recipients saw improvements in insulin sensitivity and an enrichment of their microbiota, including among those clostridial species. But six months after the transplant the patients had relapsed, metabolic improvements had faded and their microbes had reverted to their original states.

To Sonnenburg, this outcome suggests that the dance between human host and microbial community has considerable momentum. Removing the "diseased" ecosystem and installing a new one may not overcome the inertia. The gut immune system may simply mold the new community in the image of the old. That may explain why fecal transplants, which effectively vanquish *C. difficile*–associated diarrhea, have so far failed to treat inflammatory bowel disease. The former is caused by a single opportunist; the latter may be driven by an out-of-whack ecosystem and our response to the microbial derangement.

To overcome the inertia, Sonnenburg foresees treating the host *and* the microbiota simultaneously. The idea has not been tested, but he imagines clearing out the microbiota, perhaps with antibiotics, followed by immunosuppressants to quiet the patient's immune system and allow healing. Only then might the new community of microbes stick and successfully recalibrate the immune system.

EVOLUTION OF MOBILITY

WHEN ANIMAL LIFE EXPLODED some 800 million years ago, microbes had already existed on Earth for maybe three billion years. A major innovation in animal evolution was the gut—a tube that takes nutrients in one end and expels waste from the other. It is even possible, argues Margaret McFall-Ngai, a microbiologist at the University of Wisconsin–Madison, that microbes drove the evolution of the gut directly. Plants only succeeded in colonizing land when they had developed relationships with microbes that helped them extract vital nutrients from soil. Perhaps one evolutionary innovation of animals was to scoop up the microbial communities necessary for survival and to take them along for the ride, achieving mobility.



DANGEROUS OPPORTUNIST: Bilophila wadsworthia, *a species of bacterium linked to inflammatory bowel disease, bloomed in the microbiota of human volunteers fed a high-fat, high-protein diet in a recent experiment.*

Mucus may be one way the human gut selects for these microbes. Only co-adapted bacteria, Sonnenburg thinks, can metabolize the complex sugars it contains. A cornerstone of this symbiosis may be the simple imperative of acquiring nutrients in a world of scarcity. We hunt and gather the goods; the microbes ferment what we cannot digest, taking a cut in the process and keeping pathogens at bay. Our immune systems quiet down when they receive signals, conveyed partly in microbial metabolites, indicating that the right microbes are in place.

The field of gut microbiome research has already moved from the idea of describing the core species to identifying the core ecological functions various microbes perform. Many potential species may fulfill any given role. Now another concept may be emerging, which might be called the keystone relationship. "The interaction between fiber and microbes that consume it," Sonnenburg says, "is the fundamental keystone interaction that everything else is built on in the gut." It may lie at the heart of the symbiotic pact between microbes and humans.

Moises Velasquez-Manoff *is author of* An Epidemic of Absence: A New Way of Understanding Allergies and Autoimmune Diseases. *His work has appeared in the* New York Times, Mother Jones *and* Nautilus.

THE NOTION THAT THE STATE of our gut governs our state of mind dates back more than 100 years. Many 19th- and early 20th-century scientists believed that accumulating wastes in the colon triggered a state of "auto-intoxication," whereby poisons emanating from the gut produced infections that were in turn linked with depression, anxiety and psychosis. Patients were treated with colonic purges and even bowel surgeries until these practices were dismissed as quackery.

MENTAL HEALTH

Thinking from the Gut

The microbiome may yield a new class of psychobiotics for the treatment of anxiety, depression and other mood disorders

By Charles Schmidt

The ongoing exploration of the human microbiome promises to bring the link between the gut and the brain into clearer focus. Scientists are increasingly convinced that the vast assemblage of microfauna in our intestines may have a major impact on our state of mind. The gut-brain axis seems to be bidirectional—the brain acts on gastrointestinal and immune functions that help to shape the gut's microbial makeup, and gut microbes make neuroactive compounds, including neurotransmitters and metabolites that also act on the brain. These interactions could occur in various ways: microbial compounds communicate via the vagus nerve, which connects the brain and the digestive tract, and microbially derived metabolites interact with the immune system, which maintains its own communication with the brain. Sven Pettersson, a microbiologist at the Karolinska Institute in Stockholm, has recently shown that gut microbes help to control leakage through both the intestinal lining and the blood-brain barrier, which ordinarily protects the brain from potentially harmful agents.

Microbes may have their own evolutionary reasons for communicating with the brain. They need us to be social, says John Cryan, a neuroscientist at University College Cork in Ireland, so that they can spread through the human population. Cryan's research shows that when bred in sterile conditions, germ-free mice lacking in intestinal microbes also lack an ability to recognize other mice with whom they interact. In other studies, disruptions of the microbiome induced mice behavior that mimics human anxiety, depression and even autism. In some cases, scientists restored more normal behavior by treating their test subjects with certain strains of benign bacteria. Nearly all the data so far are limited to mice, but Cryan believes the findings provide fertile ground for developing analogous compounds, which he calls psychobiotics, for humans. "That dietary treatments could be used as either adjunct or sole therapy for mood disorders is not beyond the realm of possibility," he says.

PERSONALITY SHIFTS

SCIENTISTS USE germ-free mice to study how the lack of a microbiome—or selective dosing with particular bacteria—alters behavior and brain function, "which is something we could never do in people," Cryan says. Entire colonies of germ-free mice are bred and kept in isolation chambers, and the technicians who handle them wear full bodysuits, as if they were in a biohazard facility. As with all mice research, extrapolating results to humans is a big step. That is especially true with germ-free mice because their brains and immune systems are underdeveloped, and they tend to be more hyperactive and daring than normal mice.

A decade ago a research team led by Nobuyuki Sudo, now a professor of internal medicine at Kyushu University in Japan, restrained germ-free mice in a narrow tube for up to an hour and then measured their stress hormone output. The amounts detected in the germ-free animals were far higher than those measured in normal control mice exposed to the same restraint. These hormones are released by the hypothalamic-pituitary-adrenal axis, which in the germ-free mice was clearly dysfunctional. But more important, the scientists also found they could induce more normal hormonal responses simply by pretreating the animals with a single microbe: a bacterium called *Bifidobacterium infantis*. This finding showed for the first time that intestinal microbes could influence stress responses in the brain and hinted at the possibility of using probiotic treatments to affect brain function in beneficial ways. "It really got the field off the ground," says Emeran Mayer, a gastroenterologist and director of the Center for Neurobiology of Stress at the University of California, Los Angeles.

Meanwhile a research team at McMaster University in Ontario led by microbiologist Premsyl Bercik and gastroenterologist Stephen Collins discovered that if they colonized the intestines of one strain of

The Diverse Microbiome of the Hunter-Gatherer

The Hadza of Tanzania offer a snapshot of the co-adaptive capacity of the gut ecosystem

By Stephanie L. Schnorr

We tend to forget that modern humanity is largely sheltered from the last vestiges of wild untamed Earth and that our way of life bears little resemblance to how our ancestors lived during 90 percent of human history. We have lost nearly all trace of these former selves—and, worse, have marginalized the few remaining humans who retain their hunter-gatherer identity. In Tanzania, tribes of wandering foragers called the Hadza, who have lived for thousands of years in the East African Rift Valley ecosystem, tell us an immense and precious story about how humans, together with their microbial evolutionary partners, are adapted to live and thrive in a complex natural environment.

Ongoing research with the Hadza to characterize the hunter-gatherer-microbiome relationship has yielded not only insight into the co-adaptive capacity of this microbial ecosystem but also a profound appreciation for how versatile human life can be. The microbiome is central to our biology. It mediates the interaction and exchange of information across host-environment thresholds such as the mouth, skin and gut.

The strength and importance of this mediation are borne out in the Hadza gut microbiota. Their microbiome harbors incredibly high taxonomic diversity, indicating great ecosystem stability and flexibility. It is capable of withstanding the perpetual presence of parasites and pathogens and can respond to fluctuations in diet caused by an unpredictable and seasonally dependent food supply. Interestingly, bacterial taxonomic abundance is different in Hadza men and women. Because of the sexual division of labor in Hadza society, men and women tend to consume more of their respective foraged food resources. The women primarily collect and eat tubers and other plant foods. As a result, it appears that women carry more bacteria to help process the plant fiber in their diets. This difference has direct implications for how the gut microbiota may enable Hadza

germ-free mice with bacteria taken from the intestines of another mouse strain, the recipient animals would take on aspects of the donor's personality. Naturally timid mice would become more exploratory, whereas more daring mice would become apprehensive and shy. These tendencies suggested that microbial interactions with the brain could induce anxiety and mood disorders.

Bercik and Collins segued into gut-brain research from their initial focus on how the microbiome influences intestinal illnesses. People who suffer from these conditions often have co-occurring psychiatric problems such as anxiety and depression that cannot be fully explained as an emotional reaction to being sick. By colonizing germ-free mice with the bowel contents of people with irritable bowel syndrome, which induces constipation, diarrhea, pain and low-grade inflammation but has no known cause, the McMaster's team reproduced many of the same gastrointestinal symptoms. The animals developed leaky intestines, their immune systems activated, and they produced a barrage of pro-inflammatory metabolites, many with known nervous system effects. Moreover, the mice also displayed anxious behavior, as indicated in a test of their willingness to step down from a short raised platform.

AUTISM CONNECTION?

SCIENTISTS HAVE ALSO BEGUN to explore the microbiome's potential role in autism. In 2007 the late Paul Patterson, a neuroscientist and developmental biologist at the California Institute of Technology, was intrigued by epidemiological data showing that women who suffer from a high, prolonged fever during pregnancy are up to seven times more likely to have a child with autism. These data suggested an alternative cause for autism besides genetics. To investigate, Patterson induced flu-like symptoms in pregnant mice with a viral mimic: an immunostimulant called polyinosinic:polycytidylic acid, or poly(I:C). He called this the maternal immune activation (MIA) model.

The offspring of Patterson's MIA mice displayed all three of the core features of human autism: limited social interactions, a tendency toward repetitive behavior and reduced communication, which he assessed by using a special microphone to measure the length and duration of their ultrasonic vocalizations. In addition, the mice had leaky intestines, which was important because anywhere from 40 to 90 percent of all children with autism suffer from gastrointestinal symptoms.

Then Caltech microbiologist Sarkis Mazmanian and his doctoral student Elaine Hsiao discovered that MIA mice also have abnormal microbiomes. Specifically, two bacterial classes—Clostridia and Bacteroidia—were far more abundant in the MIA offspring than in normal mice. Mazmanian acknowledges that these imbalances may not be the same as those in humans with autism. But the finding was compelling, he says, because it suggested that the behavioral state of the MIA mice—and perhaps by extension autistic behavior in humans—might be rooted in the gut rather than the brain. "That raised a provocative question," Mazmanian says. "If we treated gastrointesti-

women to obtain adequate nutrition for fertility and reproductive success, despite a resource-limited environment. Through our work with the Hadza, we have been able to contribute to mounting evidence that human microbiota exerts a powerful influence on host health and survival, especially in natural fertility- and subsistence-based populations.

Comparative analysis of the gut microbiota of hunter-gatherers with that of Westernized industrial populations is also beginning to yield important insights. The microbial diversity in industrial groups is far below that of the Hadza, as well as those of other rural farming communities in Burkina Faso, Malawi and South Africa. Whereas a reduction in diversity may not seem ideal, it is the predictable response of an ecosystem facing a narrow range of selective pressures and is therefore no less adaptive. Some technological interventions, such as hypersanitation, consumption of refined foods and habitual use of antibiotics, have had a dramatic impact over time on the functional role of the microbiome in industrial populations. These aspects of a Westernized way of life have to a large extent displaced much of the original mutualistic functions of the microbiome in stabilizing our bodies against foreign microorganisms, allowing us to digest unprocessed foods and helping train our immune system to effectively fight disease.

We are just beginning to understand how the microbiome evolves over our lifetimes as a dynamic and mutualistic ecosystem that helps to facilitate human health. Thanks to the Hadza, we know that ancient human hunter-gatherers must have maintained a direct and persistent interface with the natural environment. As a result, the ancestral human microbiome was almost certainly a taxonomically diverse community, providing the functional flexibility that accompanied global colonization and is our adaptive legacy.

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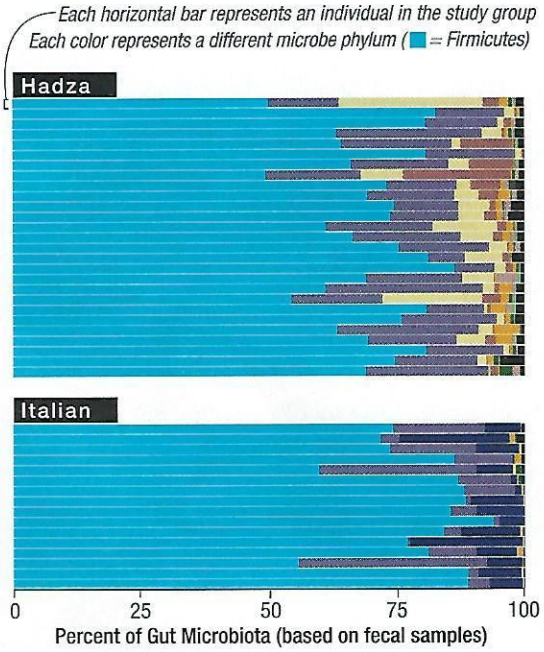

TAXONOMIC TREASURE TROVE:
A survey of fecal microbiota of 43 subjects revealed a more varied mix of gut bacteria phyla among Hadza hunter-gatherers compared with urban Italians.

nal symptoms in the mice, would we see changes in their behavior?"

Mazmanian and Hsiao investigated by dosing the animals with a microbe known for its anti-inflammatory properties, *Bacteroides fragilis,* which also protects mice from experimentally induced colitis. Results showed that the treatment fixed intestinal leaks and restored a more normal microbiota. It also mitigated the tendency toward repetitive behavior and reduced communication. Mazmanian subsequently found that *B. fragilis* reverses MIA deficits even in adult mice. "So, at least in this mouse model, it suggests features of autism aren't hardwired—they're reversible—and that's a huge advance," he says.

LIMITS OF RESEARCH

THE HUMAN GUT MICROBIOME evolved to help us in myriad ways: Gut microbes make vitamins, break dietary fiber into digestible short-chain fatty acids and govern normal functions in the immune system. Probiotic treatments such as yogurt supplemented with beneficial strains of bacteria are already being used to help treat some gastrointestinal disorders, such as antibiotic-induced diarrhea. But there are little data about probiotic effects on the human brain.

In a proof-of-concept study Mayer and his colleagues at U.C.L.A. uncovered the first evidence that probiotics ingested in food can alter human brain function. The researchers gave healthy women yogurt twice a day for a month. Then brain scans using functional magnetic resonance imaging were taken as the women were shown pictures of actors with frightened or angry facial expressions. Normally, such images trigger increased activity in emotion-processing areas of the brain that leap into action when someone is in a state of heightened alert. Anxious people may be uniquely sensitive to these visceral reactions. But the women on the yogurt diet exhibited a less "reflexive" response, "which shows that bacteria in our intestines really do affect how we interpret the world," says gastroenterologist Kirsten Tillisch, the study's principal investigator. Mayer cautions that the results are rudimentary. "We simply don't know yet if probiotics will help with human anxiety," he says. "But our research is moving in that direction."

Strains of *Bifidobacterium,* which is common in the gut flora of many mammals, including humans, have generated the best results so far. Cryan recently published a study in which two varieties of *Bifidobacterium* produced by his lab were more effective than escitalopram (Lexapro) at treating anxious and depressive behavior in a lab mouse strain known for pathological anxiety. Although Cryan is optimistic that such findings may point the way to the development of psychobiotics, he is wary of hype. "We still need a lot more research into the mechanisms by which gut bacteria interact with the brain," he says.

Charles Schmidt *is a recipient of the National Association of Science Writers' Science in Society Journalism Award. His work has appeared in* Science, Nature Biotechnology, Nature Medicine *and the* Washington Post.

Graphic by Jen Christiansen