

THE ULTIMATE SOCIAL NETWORK.

By: Jennifer Ackerman, Scientific American, Jun 2012, Vol. 306, Issue 6

Researchers who study the friendly bacteria that live inside all of us are starting to sort out who is in charge -- microbes or people?

BIOLOGISTS ONCE THOUGHT THAT HUMAN BEINGS WERE physiological islands, entirely capable of regulating their own internal workings. Our bodies made all the enzymes needed for breaking down food and using its nutrients to power and repair our tissues and organs. Signals from our own tissues dictated body states such as hunger or satiety. The specialized cells of our immune system taught themselves how to recognize and attack dangerous microbes -- pathogens -- while at the same time sparing our own tissues.

Over the past 10 years or so, however, researchers have demonstrated that the human body is not such a neatly self-sufficient island after all. It is more like a complex ecosystem -- a social network -- containing trillions of bacteria and other microorganisms that inhabit our skin, genital areas, mouth and especially intestines. In fact, most of the cells in the human body are not human at all. Bacterial cells in the human body outnumber human cells 10 to one. Moreover, this mixed community of microbial cells and the genes they contain, collectively known as the microbiome, does not threaten us but offers vital help with basic physiological processes -- from digestion to growth to self-defense.

So much for human autonomy. Biologists have made good progress characterizing the most prevalent species of microbes in the body. More recently, they have begun to identify the specific effects of these residents. In so doing, they are gaining a new view of how our bodies function and why certain modern diseases, such as obesity and autoimmune disorders, are on the rise.

OUT OF MANY, ONE

WHEN PEOPLE THINK of microbes in the body, they usually think of pathogens. Indeed, for a long time researchers focused solely on these harmful bugs and ignored the possible importance of more benign ones. The reason, argues biologist Sarkis K. Mazmanian of the California Institute of Technology, is our skewed view of the world. "Our narcissism held us back; we tended to think we had all the functions required for our health," he says. "But just because microbes are foreign, just because we acquire them throughout life, doesn't mean they're any less a fundamental part of us."

Indeed, all humans have a microbiome from very early in life, even though they do not start out with one. Each individual acquires his or her own community of commensals (from the Latin for "sharing a table") from the surrounding environment. Because the womb does not normally contain bacteria, newborns begin life as sterile, singular beings. But as they pass through the birth canal, they pick up some of Mom's commensal cells, which then begin to multiply. Breastfeeding and handling by proud parents, grandparents, siblings, and friends -- not to mention ordinary contact with bedsheets, blankets, and even pets -- quickly contribute to an expanding ark of microbes. By late infancy our bodies support one of the most complex microbial ecosystems on the planet.

For the past five years or so scientists have been working to characterize the nature of this ecosystem. The task has been devilishly difficult. The bacterial cells in the intestines, for example, have evolved to

grow in the crowded, oxygen-free environment of the gut, so many species do not survive well in the lonely expanse of a petri dish. Researchers have gotten around this problem, however, by studying the genetic instructions, the strands of DNA and RNA, found within a microbe rather than the whole cell itself. Because DNA and RNA can be manipulated in a normal, oxygenated laboratory environment, investigators can take microbial samples from the body, extract the genomic material and analyze the results.

Each species of commensal bacteria has a signature, it turns out -- its own unique version of a gene (known as the 16S ribosomal RNA gene) that codes for a particular RNA molecule found in the ribosomes, the protein-making machinery of cells. By determining the sequence of this gene, scientists are creating a catalogue of the entire human microbiome. In this way, they can glean which species exist in our bodies and how the precise combination of species may differ from one person to another.

The next step is to analyze other genes found in the microbial community to determine which ones are active in people and what functions they perform. Again, that chore is a tall order because of the great number of species and because their genes get mixed together in the extraction process. Determining whether a specific bacterial gene is active (or expressed) in the body is relatively straightforward; figuring out to which species that particular gene belongs is not. Fortunately, the development of ever more powerful computers and ultrafast gene sequences in the first decade of the 21st century has turned what would once have been an impossible task of sorting and analysis into merely a very complicated one.

Two separate groups of scientists, one in the U.S. and the other in Europe, have harnessed this new technology to enumerate the bacterial genes within the human body. In early 2010 the European group published its census of microbial genes in the human digestive system -- 3.3 million genes (from more than 1,000 species) -- about 150 times the 20,000 to 25,000 genes in the human genome.

Research into the nature of the human microbiome has yielded many surprises: no two people share the same microbial makeup, for instance -- even identical twins. This finding may help unravel a mystery presented by the Human Genome Project, which confirmed that the human DNA of all people the world over is 99.9 percent alike. Our individual fates, health and perhaps even some of our actions may have much more to do with the variation in the genes found in our microbiome than in our own genes. And although the microbiomes of different people vary markedly in the relative number and types of species they contain, most people share a core complement of helpful bacterial genes, which may derive from different species. Even the most beneficial bacteria can cause serious illness, however, if they wind up somewhere they are not supposed to be -- for example, in the blood (causing sepsis) or in the web of tissue between the abdominal organs (causing peritonitis).

FRIENDS WITH BENEFITS

THE FIRST INKLING that beneficial bugs might do us good came decades ago during research on digestion and the production of vitamins in the guts of animals. By the 1980s investigators had learned that human tissue needs vitamin B12 for, among other things, cellular energy production, DNA synthesis and the manufacture of fatty acids and had determined that only bacteria synthesize the enzymes needed to make the vitamin from scratch. Similarly, scientists have known for years that gut bacteria break down certain components of food that would otherwise be indigestible and would pass

out of the body unused. Only in the past few years, however, have they learned the juicy details: two commensal species in particular play major roles in both digestion and the regulation of appetite.

Perhaps the prime example of a helpful bug sounds like it was named after a Greek sorority or fraternity. *Bacteroides thetaiotaomicron* is a champion carbohydrate chomper, capable of breaking down the large, complex carbohydrates found in many plant foods into glucose and other small, simple, easily digestible sugars. The human genome lacks most of the genes required to make the enzymes that degrade these complex carbohydrates. *B. thetaiotaomicron*, on the other hand, has genes that code for more than 260 enzymes capable of digesting plant matter, thus providing humans with a way to efficiently extract nutrients from oranges, apples, potatoes and wheat germ, among other foods.

Fascinating details about how *B. thetaiotaomicron* interacts with, and provides sustenance to, its hosts come from studies of mice raised in a completely sterile environment (so they had no microbiome) and then exposed only to this particular strain of microbes. In 2005 researchers at Washington University in St. Louis reported that *B. thetaiotaomicron* survives by consuming complex carbohydrates known as polysaccharides. The bacteria ferment these substances, generating short-chain fatty acids (essentially their feces) that the mice can use as fuel. In this way, bacteria salvage calories from normally indigestible forms of carbohydrate, such as the dietary fiber in oat bran. (Indeed, rodents that are completely devoid of bacteria have to eat 30 percent more calories than do rodents with an intact microbiome to gain the same amount of weight.)

The study of the microbiome has even partially rehabilitated the reputation of one disease-causing bacterium called *Helicobacter pylori*. Fingerprinted by Australian physicians Barry Marshall and Robin Warren in the 1980s as the causative agent of peptic ulcers, *H. pylori* is one of the few bacteria that seem to thrive in the acidic environment of the stomach. While continued use of medicines known as nonsteroidal anti-inflammatory drugs, or NSAIDs, had long been known to be a common cause of peptic ulcers, the finding that bacteria contributed to the condition was remarkable news. After Marshall's discovery, it became standard practice to treat peptic ulcers with antibiotics. As a result, the rate of *H. pylori*-induced ulcers has dropped by more than 50 percent.

Yet the matter is not so simple, says Martin Blaser, now a professor of internal medicine and microbiology at New York University who has studied *H. pylori* for the past 25 years. "Like everyone, I started working on *H. pylori* as a simple pathogen," he says. "It took a few years for me to realize that it was actually a commensal." In 1998 Blaser and his colleagues published a study showing that in most people, *H. pylori* benefits the body by helping to regulate levels of stomach acids, thus creating an environment that suits itself and its host. If the stomach churns out too much acid for the bacteria to thrive, for example, strains of the bug that contain a gene called *cagA* start producing proteins that signal the stomach to tone down the flow of acid. In susceptible people, however, *cagA* has an unwelcome side effect: provoking the ulcers that earned *H. pylori* its nasty rap.

A decade later Blaser published a study suggesting that *H. pylori* has another job besides regulating acid. For years scientists have known that the stomach produces two hormones involved in appetite: ghrelin, which tells the brain that the body needs to eat, and leptin, which -- among other things -- signals that the stomach is full and no more food is needed. "When you wake up in the morning and you're hungry, it's because your ghrelin levels are high," Blaser says. "The hormone is telling you to eat. After you eat

breakfast, ghrelin goes down," which scientists refer to as a postprandial (from the Latin word *prandium*, for "a meal") decrease.

In a study published last year, Blaser and his colleagues looked at what happens to ghrelin levels before and after meals in people with and without *H. pylori*. The results were clear: "When you have *H. pylori*, you have a postprandial decrease in ghrelin. When you eradicate *H. pylori*, you lose that," he says. "What that means, a priori, is that *H. pylori* is involved in regulating ghrelin" -- and thus appetite. How it does so is still largely a mystery. The study of 92 veterans showed that those treated with antibiotics to eliminate *H. pylori* gained more weight in comparison to their uninfected peers -- possibly because their ghrelin level stayed elevated when it should have dropped, causing them to feel hungry longer and to eat too much.

Two or three generations ago more than 80 percent of Americans played host to the hardy bug. Now less than 6 percent of American children test positive for it. "We have a whole generation of children who are growing up without *H. pylori* to regulate their gastric ghrelin," Blaser says. Moreover, children who are repeatedly exposed to high doses of antibiotics are likely experiencing other changes in their microbial makeup. By the age of 15, most children in the U.S. have had multiple rounds of antibiotic treatment for a single ailment -- otitis media, or ear infection. Blaser speculates that this widespread treatment of young children with antibiotics has caused alterations in the compositions of their intestinal microbiome and that this change may help explain rising levels of childhood obesity. He believes that the various bacteria within the microbiome may influence whether a certain class of the body's stem cells, which are relatively unspecialized, differentiate into fat, muscle or bone. Giving antibiotics so early in life and thereby eliminating certain microbial species, he argues, interferes with normal signaling, thereby causing overproduction of fat cells.

Could the accelerating loss of *H. pylori* and other bacteria from the human microbiome, along with societal trends -- such as the easy availability of high-calorie food and the continuing decline in manual labor -- be enough to tip the balance in favor of a global obesity epidemic? "We don't know yet whether it's going to be a major or minor part of the obesity story, " he says, "but I'm betting it's not trivial."

The widespread use of antibiotics is not the only culprit in the unprecedented disruption of the human microbiome in Blaser's view. Major changes in human ecology over the past century have contributed as well. The dramatic increase in the past few decades in the number of deliveries by cesarean section obviously limits the transfer through the birth canal of those all-important strains from Mom. (In the U.S., more than 30 percent of all newborns are delivered by C-section, and in China -- land of one child per couple -- the operation is responsible for nearly two thirds of all births to women living in urban areas.) Smaller family sizes throughout the world mean fewer siblings, who are a prime source of microbial material to their younger siblings during early childhood years. Even cleaner water -- which has saved the lives of untold millions -- exacts a toll on the human microbiome, reducing the variety of bacteria to which we are exposed. The result: more and more people are born into and grow up in an increasingly impoverished microbial world.

A DELICATE BALANCE

AS THE ONGOING STUDIES of *B. thetaiotaomicron* and *H. pylori* illustrate, even the most basic questions about what these bacterial species are doing in the body lead to complicated answers. Going

one step further and asking how the body responds to the presence of all these foreign cells in its midst introduces even greater complexity. For one thing, the traditional understanding of how the immune system distinguishes the body's own cells (self) from genetically different cells (nonself) suggests that our molecular defenses should be in a constant state of war against these myriad interlopers. Why the intestines, for example, are not the scene of more pitched battles between human immune cells and the trillions of bacteria present is one of the great, as yet unsolved mysteries of immunology.

The few clues that exist offer tantalizing insights into the balancing act between the microbiome and human immune cells that has taken some 200,000 years to calibrate. Over the eons the immune system has evolved numerous checks and balances that generally prevent it from becoming either too aggressive (and attacking its own tissue) or too lax (and failing to recognize dangerous pathogens). For example, T cells play a major role in recognizing and attacking microbial invaders of the body, as well as unleashing the characteristic swelling, redness and rising temperature of a generalized inflammatory response to infection by a pathogen. But soon after the body ramps up its production of T cells, it also starts producing so-called regulatory T cells, whose principal function seems to be to counteract the activity of the other, pro-inflammatory T cells.

Normally the regulatory T cells swing into action before the pro-inflammatory T cells get too carried away. "The problem is that many of the mechanisms that these proinflammatory T cells use to fight infection -- for example, the release of toxic compounds -- end up blasting our own tissues," says Caltech's Mazmanian. Fortunately, the regulatory T cells produce a protein that restrains the proinflammatory T cells. The net effect is to tamp down inflammation and prevent the immune system from attacking the body's own cells and tissues. As long as there is a good balance between belligerent T cells and more tolerant regulatory T cells, the body remains in good health.

For years researchers assumed that this system of checks and balances was generated entirely by the immune system. But in yet another example of how little we control our own fate, Mazmanian and others are starting to show that a healthy, mature immune system depends on the constant intervention of beneficial bacteria. "It goes against dogma to think that bacteria would make our immune systems function better," he says. "But the picture is getting very clear: the driving force behind the features of the immune system are commensals."

Mazmanian and his team at Caltech have discovered that a common microorganism called *Bacteroides fragilis*, which lives in some 70 to 80 percent of people, helps to keep the immune system in balance by boosting its anti-inflammatory arm. Their research began with observations that germ-free mice have defective immune systems, with diminished function of regulatory T cells. When the researchers introduced *B. fragilis* to the mice, the balance between the pro-inflammatory and anti-inflammatory T cells was restored, and the rodents' immune systems functioned normally.

But how? In the early 1990s researchers started characterizing several sugar molecules that protrude from the surface of *B. fragilis* -- and by which the immune system recognizes its presence. In 2005 Mazmanian and his colleagues showed that one of these molecules, known as polysaccharide A, promotes maturation of the immune system. Subsequently, his laboratory revealed that polysaccharide A signals the immune system to make more regulatory T cells, which in turn tell the pro-inflammatory T cells to leave the bacterium alone. Strains of *B. fragilis* that lack polysaccharide A simply do not survive in the mucosal lining of the gut, where immune cells attack the microbe as if it were a pathogen.

In 2011 Mazmanian and his colleagues published a study in *Science* detailing the full molecular pathway that produces this effect -- the first such illumination of a molecular pathway for mutualism between microbe and mammal. "*B. fragilis* provides us with a profoundly beneficial effect that our own DNA for some reason doesn't provide," Mazmanian says. "In many ways, it co-opts our immune system -- hijacks it." Unlike pathogens, however, this hijacking does not inhibit or reduce our immune system performance but rather helps it to function. Other organisms may have similar effects on the immune system, he notes: "This is just the first example. There are, no doubt, many more to come."

Alas, because of lifestyle changes over the past century, *B. fragilis*, like *H. pylori*, is disappearing. "What we've done as a society over a short period is completely change our association with the microbial world," Mazmanian says. "In our efforts to distance ourselves from disease-causing infectious agents, we have probably also changed our associations with beneficial organisms. Our intentions are good, but there's a price to pay."

In the case of *B. fragilis*, the price may be a significant increase in the number of autoimmune disorders. Without polysaccharide A signaling the immune system to churn out more regulatory T cells, the belligerent T cells begin attacking everything in sight -- including the body's own tissues. Mazmanian contends that the recent sevenfold to eightfold increase in rates of autoimmune disorders such as Crohn's disease, type 1 diabetes and multiple sclerosis is related to the decline in beneficial microbes. "All these diseases have both a genetic component and an environmental component," Mazmanian says. "I believe that the environmental component is microbiotic and that the changes are affecting our immune system." The microbial shift that comes with changes in how we live -- including a decrease in *B. fragilis* and other anti-inflammatory microbes -- results in the underdevelopment of regulatory T cells. In people who have a genetic susceptibility, this deviation may lead to autoimmunity and other disorders.

Or at least that is the hypothesis. At this stage in the research, the correlations in humans between lower microbial infections and increased rates of immune disease are only that -- correlations. Just as with the obesity issue, teasing apart cause and effect can be difficult. Either the loss of humanity's indigenous bugs have forced rates of autoimmune diseases and obesity to shoot up or the increasing levels of autoimmunity and obesity have created an unfavorable climate for these native bugs. Mazmanian is convinced that the former is true -- that changes in the intestinal microbiome are contributing significantly to rising rates of immune disorders. Yet "the burden of proof is on us, the scientists, to take these correlations and prove that there is cause and effect by deciphering the mechanisms underlying them," Mazmanian says. "That is the future of our work."

MORE TO EXPLORE

Who Are We? Indigenous Microbes and the Ecology of Human Diseases. Martin J. Blaser in *EMBO Reports*, Vol. 7, No. 10, pages 956-960; October 2006. www.ncbi.nlm.nih.gov/pmc/articles/PMC1618379

A Human Gut Microbial Gene Catalogue Established by Metagenomic Sequencing. Junjie Qin et al. in *Nature*, Vol. 464, pages 59-65; March 4, 2010.

Has the Microbiota Played a Critical Role in the Evolution of the Adaptive Immune System? Yun Kyung Lee and Sarkis K. Mazmanian in *Science*, Vol. 330, pages 1768-1773; December 24, 2010. www.ncbi.nlm.nih.gov/pmc/articles/PMG159383

This extra credit exercise is worth 5 points and is optional.

Use the article above and other sources as needed to answer the following questions about the relationship between humans and the bacteria that live within us. **The answers to these questions must be typed and complete. Each question is not worth a point; rather I will judge the overall quality of your answers and assign points accordingly. While you will be getting your answers from other sources, use your own words to answer these questions.**

1. Humans have 10x as many bacteria as their own cells yet humans do not have these bacteria before birth. How do they acquire the enormous number of microbes throughout their bodies so quickly?
2. Bacteria are most abundant in the digestive system, particularly the lower intestine. What two important functions do bacteria have in the gut? Why are these functions adaptive for humans?
3. How can the loss of particular bacterial strains such as *Helicobacter pylori* contribute to an increased likelihood of becoming obese?
4. What role may do beneficial bacteria appear to play in the regulation of the immune system?
5. Humans in developed countries like the U.S. are exposed to far fewer bacteria than in the past. Why is this? List at least two reasons. Why is this potentially a troubling development?