

Say Hello to Your Little Friends

By Marcus Y. Woo

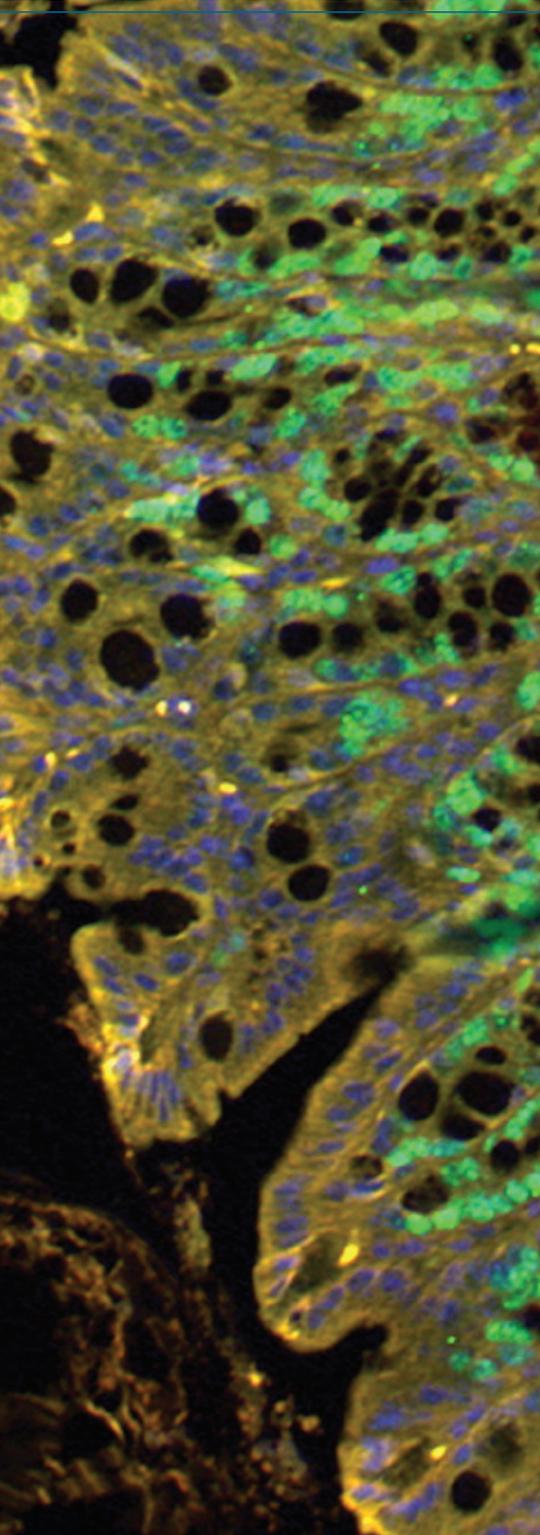
Bacteria are mostly known for making you sick. But not all microbes are malicious, and in fact, trillions of them live in your gut. It turns out that these bugs aren't just harmless, they may be crucial for your health.

Your bowels bustle with about a hundred trillion bacteria. That's 10 times more microorganisms than you have cells in your body. Put another way, if you had a buck for every bug, you could lift the U.S. federal government out of debt not once, but 10 times over. These intestinal bacteria consist of thousands of species, perhaps as many as 35,000. You may squirm at the thought of trillions of bugs crawling inside you—after all, bacteria don't exactly have a good reputation. These microscopic critters are known for causing deadly diseases, such as pneumonia, syphilis, cholera, tuberculosis, and meningitis. To counter these invisible scourges, people have developed antibiotics and an arsenal of disinfectant-laced household cleaners.

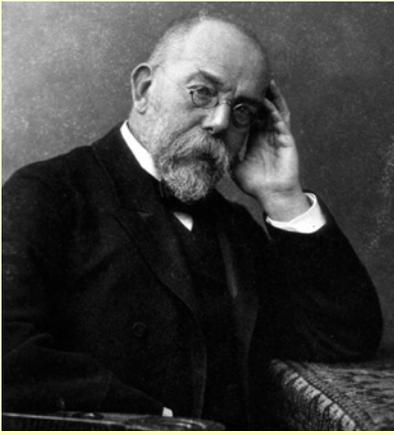
But those wayward pathogens in your bloodstream account for just a tiny fraction of bacteria. "I think we've done these organisms an injustice," says Sarkis Mazmanian, assistant professor of biology. "We group all of them as being bad, but it's really a small subset that are out to get you." The ones in your intestines are mainly harmless or even helpful. Some species assist in digestion while others monopolize the resources in

your intestinal ecosystem and prevent bad bugs from colonizing. "To put things in perspective," Mazmanian says, "there are over 60,000 different species of bacteria that are known. Only 170 of them cause diseases in mammals. Only about 90 cause disease in humans." Some microbes, namely, those dubbed probiotics, help metabolism or provide nutrients—for example, people have long turned to yogurt, which has digestion-aiding bacteria like *Bifidobacterium* and *Lactobacillus*, to settle the stomach.

But can these microbes act as more than just Maalox and multivitamins? "These are organisms that we've harbored for millions and millions of years," Mazmanian says. Because they have evolved with us, it's not hard to believe they've become an integral part of our biology. For at least two decades, scientists have been batting around the hypothesis that bacteria could keep people healthy, but the idea hasn't gained much traction for lack of evidence. "Even as recently as five years ago, it was not mainstream to talk about microbes as having benefits," Mazmanian says. But now, he and his colleagues have found that a common gut microorganism called *Bacteroides*



A cross section through a mouse colon. The tissue is stained so that immune cells that are influenced by beneficial bacteria glow green.



BAD BUGS

One of the founders of microbiology and the germ theory of disease was Robert Koch, a German physician born in 1843. In 1872, he became the district medical officer in Wollstein, a small town in present-day Poland. There, cut off from the medical community and armed with only some modest equipment in a small laboratory that doubled as his home, he experimented with anthrax, a widespread disease that was killing farm animals at the time. He confirmed earlier work by other scientists that a bacterium called *Bacillus anthracis* caused the disease. He also showed that the microbe alone—without having had prior contact with animals—was enough to trigger anthrax. Later in his career, Koch would also isolate *Vibrio cholerae* and *Mycobacterium tuberculosis*, the bacteria that lead to cholera and tuberculosis, respectively. For his work with tuberculosis, he would win the Nobel Prize in 1905.

Koch, along with others like Louis Pasteur—who developed pasteurization, the process that slows the growth of bacteria in milk and other foods—revolutionized health and hygiene. As a result, new sanitation methods and antibiotics have saved countless lives. In 1900, Americans lived, on average, around 50 years. Global life expectancy was only 30. Now, life expectancy around the world is pushing 70, and is above 80 in some countries. And the fighting continues today—the 2005 Nobel Prize in Physiology or Medicine was given to Barry Marshall and J. Robin Warren for finding that many ulcers are caused by *Helicobacter pylori*. **ES**

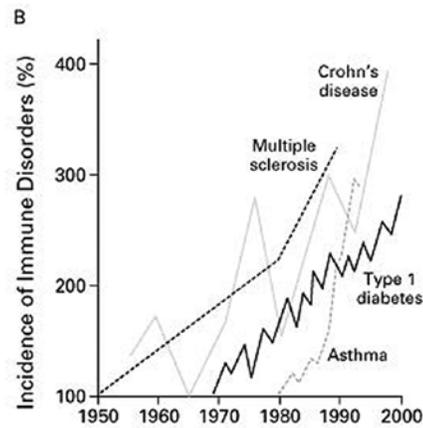
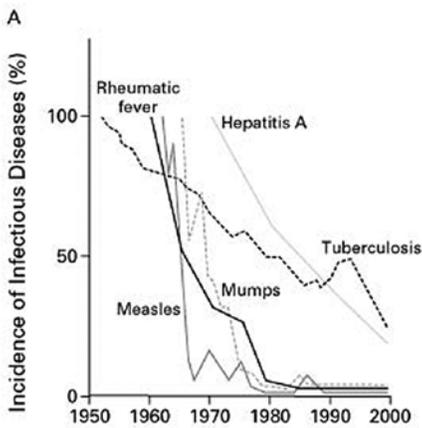
fragilis prevents inflammatory bowel disease in mice—a seminal piece of evidence that shows bacteria boosting the immune system. These findings are spurring other researchers to study beneficial bacteria, revamping our perception of the microbial world. In recognition of his work, Mazmanian has been named as one of *Discover* magazine's "20 Best Brains under 40," a list of innovative scientists around the country who are changing their fields.

Inflammatory bowel disease, also called IBD, is a group of disorders whose symptoms include abdominal cramps, diarrhea, intestinal bleeding, and weight loss. Affecting about one million Americans, according to the Crohn's and Colitis Foundation of America, IBD is one of a handful of immunological disorders—chronic ailments that arise when the immune system goes awry—including asthma, type-1 diabetes, and multiple sclerosis. If *B. fragilis* inhibits IBD, could it—or some other bug—mitigate those other diseases? No one knows yet how tiny bugs in the gut could so profoundly affect the rest of the body, and there's no evidence that bacteria cause any of these diseases. On the other hand, recent data have pointed to a puzzling pattern that suggests gut bacteria may be more important than scientists have realized.

Despite our improved overall health over the last century, immunological diseases have increased in recent decades, particularly in developed countries—places with superior health and sanitation systems. In a 2004 report, the Global Initiative for Asthma

estimated that 300 million suffer from asthma around the world and 100 million more could become asthmatic by 2025. The report also noted that asthma increases as countries become more westernized and urbanized. Of course, the exact cause of asthma is a complex issue in itself (see *E&S* 2008, No. 3), and much of the increase might be attributable to rising air pollution. But studies show that other immunological diseases have also been skyrocketing. Multiple sclerosis increased by more than 300 percent from 1950 to 1990. Crohn's disease, one of the main forms of IBD, has gone up 400 percent in the last 50 years. Type 1 diabetes has risen by almost 300 percent since 1970. Meanwhile, infectious diseases caused by pathogenic bacteria are falling.

In 1989, an epidemiologist named David P. Strachan published a paper in the *British Medical Journal* proposing the so-called hygiene hypothesis. From studying hay-fever and eczema incidences in British children, he found that those from big families were less likely to develop the diseases—both of which are immunological. With more kids running around, bacteria get swapped like baseball cards. The immune system gets lots of exercise, and people harbor a more diverse population of bacteria. Was our overall improved hygiene as a society contributing to the proliferation of allergic diseases? In the past few years researchers have begun to find supporting evidence for Strachan's hypothesis, showing that a drop in gut bacteria accompanies the rise in



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In the last half of the 20th century, infectious diseases dropped while immune disorders skyrocketed.

immunological disorders. Although no one has yet been able to show that one causes the other, scientists like Mazmanian think it's more than just a coincidence.

THE "FORGOTTEN ORGAN"

Autoimmune diseases strike when the immune system gets out of balance. The body is quite adept at ridding itself of nasty invaders, but our defensive system has its flaws. Instead of killing pathogens with precise, laser-guided missiles, the immune system might attack an entire area—or even the whole body. "The system realizes there's

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something to kill, and it just throws every weapon at it," Mazmanian says, and healthy cells often become casualties of war. Running a temperature helps roast pathogens to death, but your entire body suffers as a result, and a prolonged fever can become life-threatening. Alternatively, the immune system may respond locally to an invader, but continue to attack even after the enemy has been eradicated. Sepsis is a menace in intensive care units—the immune system carpet bombs the entire body and everything becomes inflamed. The mortality rate ranges from 30 to 50 percent, and in cases of septic shock, the rate can be even higher.

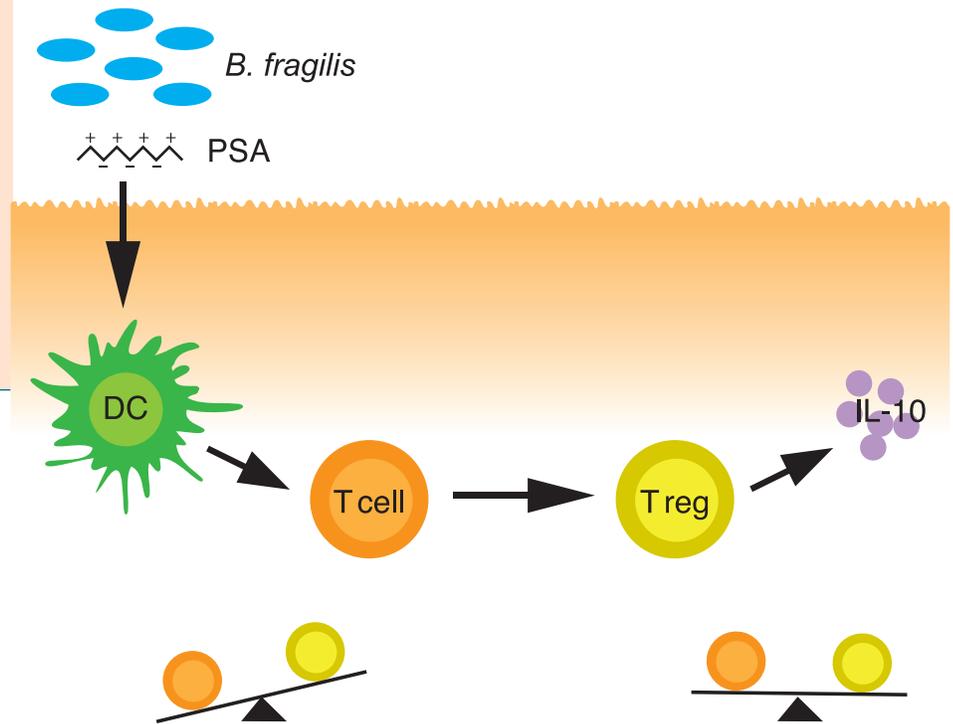
In a healthy immune system, what Mazmanian calls the anti-inflammatory arm stops the system from damaging too many healthy cells. He and his colleagues discovered that *B. fragilis*, which he says lives in roughly 70 to 80 percent of us, helps regulate this. It turns out that if the microbe is missing, the gut becomes susceptible to IBD.

Mazmanian and colleagues first began research by raising mice from birth with no gut bacteria at all. Without their bacterial fauna—called the microbiota—the mice had deficient immune systems. Their lymph nodes and spleen follicles, which filter blood, were underdeveloped, and they had an imbalance of the immune cells called Th1 and Th2 helper cells. But when *B. fragilis* was introduced, these problems disappeared; the immune system healed itself. A sugar molecule—a polysaccharide called PSA, produced by the bacteria—turned out to be responsible. When given *B. fragilis*

engineered to lack PSA, the mice didn't recover. The researchers also saw that PSA pumped up the production of an important family of immune cells called CD4 T cells, but nobody knew what this meant until later.

Published in 2005, these results were only general observations. Still, they allowed Mazmanian to ask the next question: can *B. fragilis* and PSA actually prevent or cure IBD? As Mazmanian points out, just because a roof looks like it has holes in it, you don't know for sure if it'll leak until it rains. So the researchers decided to seed the clouds. A microbe called *Helicobacter hepaticus* triggers an illness in mice that's reminiscent of Crohn's disease. The researchers used the bug to give mice IBD, and those colonized with *B. fragilis* and those injected with just PSA remained disease free. But mice that were colonized with the strains of *B. fragilis* that lacked PSA did get sick, showing that PSA was the crucial ingredient in preventing IBD. PSA molecules are captured by dendritic cells, scouts that scour the body for pathogens. The dendritic cells then tell the CD4 T cells mentioned above to become a more specialized cell subtype called the regulatory T cell. These cells produce anti-inflammatory IL-10 molecules. These molecules, small proteins called cytokines, are one of the ways the immune system stops inflammation. Without PSA, CD4 T cells can also become inflammation-inducing helper cells called Th1, Th2, and Th17. In particular, Th17 makes an important pro-inflammatory cytokine called IL-17. Thus PSA restores the balance between

B. fragilis in the gut alleviates inflammation in mice by restoring a balance of immune cells. The microbe produces a sugar molecule called PSA. A scout-like cell called a dendritic cell (green) captures PSA and tells a T cell (orange) to become a specialized subtype called a regulatory T cell (yellow). Regulatory T cells produce a molecule called IL-10 that stops inflammation.



the pro- and anti-inflammatory arms of the immune system.

“Most people believe—and it’s probably or partially true—that the absence of regulation comes from genetics,” Mazmanian says. “We and a handful of other laboratories believe that certain bacteria are actually inducing those regulatory cells.” If those bacteria are missing, the immune system becomes unbalanced, leading to the chronic inflammation behind IBD and other immunological disorders. The researchers call the gut’s bacteria the “forgotten organ”—not just a mass of microbes that have tagged along for the ride, but an important part of our bodies. “I don’t view any beneficial functions from these organisms as functions that we’ve turned over to them with time. I think they’ve been supplying these functions throughout our evolution.”

The next step is to see if *B. fragilis* actually helps maintain a healthy immune system. “Is this the default?” Mazmanian says. “Is good health a result of the bacteria constantly sending these signals?” Experiments involving germfree mice are hardly realistic. The next set of experiments will involve regular mice with a complete set of microbiota, and the lab has already started the effort. But with thousands of species of bugs in the bowel, it won’t be easy to isolate the effects of individual ones.

Says Mazmanian, “*B. fragilis* has had millions of years to figure out which molecules work and which don’t, and how it’s going to deliver them and in what doses—things that we think about now in terms of medicine, of

giving the right compound in the right way at the right time and at the right dose. But the bug has already figured this out, so we’re trying to understand what the bug already knows, and to use that, to harness that, to exploit that as a therapy.” In other words, the researchers want to reverse-engineer evolution’s experiment.

LET’S GET DIRTY?

The first two years of a baby’s life could be crucial. A fetus is germfree, but once it enters the world, bacteria begin to colonize the newborn’s intestinal tract. After a couple of years, an infant’s microbiota is the same as an adult’s. “My leading theory is that everything happens in the first two or three years of life,” Mazmanian says. “It puts you on a trajectory toward a balanced immune system or an inflamed immune system.”

Which brings us back to the hygiene hypothesis. Our cleaner world has undoubtedly reduced infectious diseases, but at the same time, it’s possible that our intestinal tract is not being colonized with the same

bacteria we’ve had for millions of years. “What we’ve done as a society, over a short period of time, is completely change our association with the microbial world,” Mazmanian says. “Our intention—the impetus—was to reduce pathogens, but antibacterial soaps, antibiotics, hygiene, and sanitation don’t discriminate between bad bacteria and good ones. These organisms are just as sensitive to antibiotics as the *Strep* that gets in your throat.”

Breeding germfree humans to replicate Mazmanian’s mouse experiments has obvious drawbacks, but researchers have started to look for correlations between immunological diseases, such as IBD and asthma, and the patients’ microbiota. Many years of measuring bacteria in infants, children, and adults may point to some immunological predictors—say, excesses of various inflammatory molecules—for these disorders. Recent studies have shown that people suffering from IBD harbor fewer microorganisms in their guts, according to Mazmanian. But because these studies are only snapshots in time, you can’t distin-

guish cause from effect—either species of *Bacteroides* were somehow lost and then IBD ensued, or IBD created an environment in the immune system that's hostile to the bugs.

So far, the scientific community is still hesitant to fully accept that tiny organisms in your intestines could affect far-removed parts of the body like the lungs or the nervous system, according to Mazmanian. These ideas are highly speculative, but given recent progress in demonstrating just how helpful bacteria can be, they're not necessarily that wild. Consider *B. fragilis*'s possible far-reaching influence—the dendritic cells could carry the PSA molecules elsewhere in the body to stop inflammation, while the microbe itself remains in the gut. "It's going against dogma to think that bacteria would help our immune system develop," he says. "Maybe it's because as humans, we're narcissists—we think we have everything we need."

In 2008, the National Institutes of Health started the Human Microbiome Project. The five-year endeavor seeks to compile the entire human microbiota in order to assess its role in health and disease. As part of the program, researchers will sequence 600 genomes of bacteria, and, in conjunction with other efforts, will put together a database of 1,000 genomes.

TRILLIONS OF LITTLE FRIENDS

The Th17 helper cell that makes the pro-inflammatory IL-17 molecule and gives rise

to IBD also happens to drive asthma, type-1 diabetes, and multiple sclerosis. What effect might *B. fragilis* have on them? No one knows if the bug can lead to treatments for these diseases, but it's a question that begs to be answered. Meanwhile, Mazmanian is embarking on another direction that could point toward a potential treatment for colon cancer.

IBD increases cancer risk by as much as five times, and although 90 percent of IBD sufferers do not get colon cancer, the two diseases are closely related. In fact, *H. hepaticus*, the bacteria that induces IBD in mice, also gives them colon cancer. IBD constantly inflames and kills cells, prompting the body to regenerate them. Continual cell division means more mutations, heightening the chance of cancerous defects. "Constant inflammation actually feeds the cancer," Mazmanian explains. "What people have begun to realize in many types of cancer is that inflammation, though it may not be the cause, is critical for the progression." Could PSA, a natural anti-inflammatory molecule, prevent the disease from progressing?

Last year, the Damon Runyon Cancer Research Foundation awarded Mazmanian with a grant to try to find out. The private organization aims to support young researchers pursuing riskier lines of research—and linking colon cancer with bacteria certainly fits the bill. "There's no evidence at all, one way or another, that bacteria are involved in colon cancer in humans," Mazmanian says. "But just because people haven't looked doesn't mean it's not there."

Mazmanian's group will remain focused on the immune system for the foreseeable future, but his basic goal is to understand the relationship between gut bacteria and health. As biologists are learning, that relationship appears to be quite intimate. So the next time you make a toast to good health, maybe you should also give thanks to your trillions of little friends within. **es**

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