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Neuroscientist Egle Cekanaviciute found that people with multiple sclerosis have different gut microbiomes from those without the disease.

When immunity goes wrong

If the gut microbiome can trigger autoimmune diseases, can it also help to cure them? **By Eric Bender**

For Gregg Silverman, a rheumatologist at New York University, the day a woman he was treating for lupus was visited by her identical twin sister was a watershed moment. The sister was a picture of health, with a one-year-old child in her arms. Silverman's patient, meanwhile, was receiving kidney dialysis and, despite his best efforts, her condition was getting worse. "Genetics was not going to explain this difference," says Silverman. The revelation launched him on a decades-long quest to seek out other factors that drive the puzzling autoimmune disease.

Researchers investigating other autoimmune diseases have also been looking beyond genetics. In the case of type 1 diabetes, the epidemiological evidence for doing so is overwhelming, says Jayne Danska, a geneticist at the Hospital for Sick Children in Toronto, Canada. Genetics "doesn't explain why the incidence of the disease has been rising over

the last 50 years in many different countries – and it doesn't explain why the age of onset is becoming progressively earlier", she says.

Many events, including viral infections and certain foods, have long been suspected of helping to trigger autoimmune diseases, in which the body attacks its own cells. But over the past decade, new suspects have emerged – the trillions of microbes inhabiting the digestive tract. Scientists have now implicated the gut microbiome in numerous autoimmune conditions, including lupus, type 1 diabetes, rheumatoid arthritis and multiple sclerosis.

For example, in 2017, researchers at the University of California, San Francisco, compared the gut microbiomes of people with multiple sclerosis with those of healthy volunteers. This study, led by geneticist Sergio Baranzini and neuroscientist Egle Cekanaviciute, found that many bacterial species were present in very different quantities in the two groups¹. This

research "not only identified differences in microbial communities, but actually showed that they had physiological significance in a human immune-cell experimental system", says Cekanaviciute, who now studies microbiome health effects at NASA's Ames Research Center in Moffett Field, California.

When two species that were more abundant in people with multiple sclerosis were incubated in human blood cells *in vitro*, the cells' inflammatory responses climbed. Another bacterial species, whose levels were depressed in people with multiple sclerosis, stimulated anti-inflammatory cells. And when the investigators transferred a microbiome from a person with multiple sclerosis into germ-free mice (those reared to be devoid of microorganisms), "these mice got a lot sicker than mice receiving a healthy human microbiome", says Baranzini.

Scientists are trying to understand the mechanisms behind the apparent ability of the gut microbiota to trigger or to sustain autoimmune conditions. They hope to turn that knowledge into better therapies for conditions that are currently difficult to treat – perhaps even in the form of simple probiotic pills.

Molecular mimicry

Autoimmune diseases are often traced, in part, to alterations in the human leukocyte antigen (HLA) gene complex, a cornerstone of the adaptive immune system, which recognizes and remembers specific pathogens. HLA genes express proteins that present antigens to our

immune system's T and B cells. The immune cells then spot and attack dangerous intruders carrying those antigen flags. T cells and B cells are selected out to ignore the body's own cells but in autoimmune diseases this doesn't happen.

Although most T cells are trained in the thymus to ignore 'self' proteins, some are trained in the gut. "Given all the different environmental factors that come in contact with the gut, you need a lot of immune tolerance there," explains Marika Falcone, an immunologist at IRCCS Ospedale San Raffaele in Milan, Italy.

Experiments in colonizing germ-free mice with specific microbes in the gut have shown that the effects are broadcast throughout the immune system, Danska says. In turn, the immune system in the gut affects the microbes there. Biologists are exploring various routes by which the gut microbiome might help to stimulate or stop immune pro-inflammatory responses – driven either by the bacteria themselves, or by the metabolites they produce, the immune cells they train, or another mechanism.

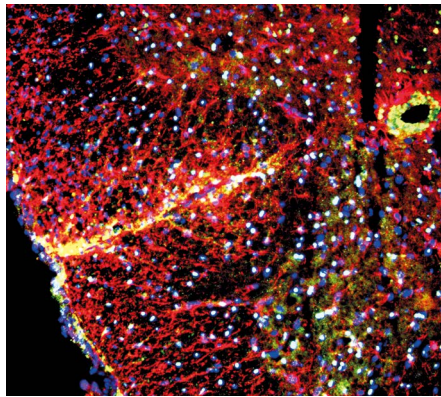
One line of enquiry is whether the enormous genetic variation between microbes leads to immune cells becoming confused as to what is foreign and what is self. A meta-analysis that examined 3,665 human samples identified more than 22 million gut microbiome genes². The proteins produced by these genes are scrutinized by the immune system, and, overwhelmingly, found to be harmless or easily handled.

But sometimes microbial proteins that alarm immune cells contain fragments that closely resemble those of normal human proteins. With roughly a hundred times as many genes in our individual microbiomes as in our own genomes, there's a high likelihood of

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similarities, says Martin Kriegel, an immunobiologist at Yale University in New Haven, Connecticut. The result, so the theory goes, is that this starts to teach the immune system to recognize human proteins as signs of a threat. In such cases of molecular mimicry, "the immune system gets confused", says Baranzini. "It starts reacting against the bacteria. And then it ends up reacting against our own self proteins."

Kriegel and his colleagues demonstrated a molecular mimicry response in cells from people with lupus using a bacterial protein very similar to the human protein Ro60 (ref. 3). Molecular mimicry could also be at work in rheumatoid arthritis – peptides produced



Micrograph of a spinal cord affected by multiple sclerosis.

by gut bacteria such as *Prevotella* closely resemble human peptides presented to the immune system in the joints of people with the condition. In a 2017 study⁴, immune reactions to the microbial peptides corresponded with those of the matching host peptides, "which was a pretty strong signal", says Allen Steere, a rheumatologist at Massachusetts General Hospital in Boston and an author of the study.

Gut to go

If gut microbiota do confuse the immune system, the question remains of how such autoimmune effects spread from the gut. In some cases, specific cells are affected, such as nerve cells in multiple sclerosis, and pancreatic β -cells in type 1 diabetes. In lupus and rheumatoid arthritis, autoimmunity occurs across multiple organs.

Steere and his colleagues found evidence of *Prevotella* DNA in the joints of some people with rheumatoid arthritis⁴. That finding, Steere says, suggests that either the bacteria themselves, or bacterial remnants carried by immune cells, can get into joints.

In the case of multiple sclerosis, "I don't think the bacteria move, but their metabolites do," says Patrizia Casaccia, a neuroscientist at the City University of New York. She notes that the metabolites might signal through the vagus nerve, which transmits messages from the gut to the brain. In some cases, bacteria themselves have been found in affected organs – such as in the pancreas in type 1 diabetes.

Kriegel and his team showed that in mice predisposed to a lupus-like condition, *Enterococcus gallinarum* bacteria move out of the gut to other organs, including the liver, where they set off an immune cascade that leads to lupus⁵. The investigators also identified similar biological pathways in human liver cells. Most importantly, Kriegel says, the bacteria were found in most liver biopsies from people with lupus – but not in those from healthy people.

His team also showed that either antibiotic treatment or a vaccine against *E. gallinarum* prevented autoimmunity developing in mice. "One could already envision a potential future therapy targeted against these bugs that cross the gut barrier," Kriegel says.

Microbes in the clinic

Reports about faecal matter transplants (FMTs) or probiotic pills have given people with immune conditions hope that there could be an easy way to prevent or treat their disease. Scientists share this desire, but warn that clinical research has barely begun.

For multiple sclerosis, for example, treatment might eventually "be as simple as a targeted dietary intervention that will shift the community from pro-inflammatory bacteria to more anti-inflammatory types", says Baranzini. In one possible step towards this goal, his team is running a small phase I clinical trial of FMT to assess safety and side effects.

Casaccia emphasizes the importance of proceeding with caution. "We want to understand the rules that regulate the bacterial society in the gut," she says. "Maybe we can develop a combination of healthy probiotics and prebiotics to support the growth of the beneficial bacteria, and perhaps dietary manipulation might contribute to that," she says. "But I'm not sure we are there yet."

Researchers do see major progress. "The enormous effort that's been spent over the last 20 years using different kinds of tools in the box is really beginning to bear fruit," says Danska. Her team has built a platform to identify antibacterial antibodies in blood. Analysing samples from children at high risk of type 1 diabetes, the platform revealed important clues about who would develop the disease⁶.

Danska hopes that better knowledge about the gut microbiome, especially during the first 3 years of life, will lead to disease-preventing interventions. Those might include giving babies well-defined compositions of microbes, so that a child's immune system "develops with optimal tolerance to self without sacrificing their ability to fight infection", she says. "That's the kind of therapy that could have global impact because bugs are cheap. If we can come up with defined compositions of microbes in a gummy bear – now we're talking!"

Eric Bender is a science writer in Newton, Massachusetts.

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