



## MICROBIOME

# Gut reaction

*Microbes are under the spotlight in efforts to unravel — and combat — allergies.*

BY CASSANDRA WILLYARD

The twists and turns of the human gut support an active and diverse microbial ecosystem. The tens of trillions of bacteria aren't just hitchhikers; they interact intimately with the immune system, and are so integral to our health that some scientists have deemed them the “forgotten organ”.

Today scientists are trying to unravel the relationship between changes in lifestyles in recent decades, changes in our microbiota, and the skyrocketing prevalence of allergies in the

developed world. Establishing a link between these phenomena could lead to treatments for allergies and asthma.

It was the ‘hygiene hypothesis’ (see ‘When allergies goes west,’ page S2) that first posited a causal link between Western lifestyles and allergy. Scientists found that zealous use of antibacterials, from cleaning products to antibiotics, had limited exposure to pathogens in early childhood. They suggested that the regulation of immune responses was compromised by this limited exposure. In the late 1990s, Agnes Wold, a bacteriologist at the

University of Gothenburg in Sweden, brought gut microbes into the equation. Wold and her colleagues observed that typical gut bacteria colonize infants in Pakistan earlier than they colonize infants in Sweden. This delay, Wold suggested, could compromise immune tolerance — affecting the ability to cope with harmless antigens such as food and pollen.

Gary Huffnagle, a microbiologist at the University of Michigan in Ann Arbor, has built on this concept of the hygiene hypothesis. He proposes that the Western lifestyle can dramatically alter our gut microflora leading to allergies and other inflammatory diseases, an idea he calls the ‘microflora hypothesis.’

The theory is supported by observational evidence. City dwellers, increasingly the predominant demographic, are exposed to a narrower range of microbes than people in rural areas — and they get more allergies. Children in rural Burkina Faso, where allergies are rare and the typical diet is high in fibre, have a different profile of microbes in their faeces than children living in Europe. The rapid increase in allergic diseases in the West has coincided with widespread use of antibiotics, especially broad-spectrum drugs. Antibiotics can profoundly alter the microbial composition of the gut, and studies show that children who are given antibiotics in their first year are more susceptible to allergies. “More and more of these smoking guns point to the role of the microbiota affecting immune development,” says Brett Finlay, a microbiologist at the Michael Smith Laboratories, University of British Columbia in Vancouver.

## BUGGING THE IMMUNE SYSTEM

The immune cells in the gut are in constant contact with a diverse microbial milieu, and the human gut “has more immune cells than the rest of the body put together,” says David Artis, a microbiologist at the University of Pennsylvania in Philadelphia. To an immune cell, beneficial or harmless bacteria (known as commensals) look much like harmful ones, but the beneficial bugs have developed methods of shaping the function of the immune system, so that their presence doesn't provoke an immune attack. “These bugs are flipping switches,” says Sarkis Mazmanian, a microbiologist at the California Institute of Technology in Pasadena. If these beneficial microbes fail to colonize our guts early in life, or if they succumb to a course of antibiotics, then switches don't get flipped and the immune system can become hypersensitive, attacking harmless microbes and other substances such as pollen, pet dander or shellfish — or so the thinking goes.

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Scientists are still trying to figure out which switches are flipped, how the commensal bacteria flip them, and

what the consequences are. “I think there will probably be multiple pathways through which commensals can influence allergic disease,” Artis says.

Several of these pathways appear to involve regulatory T cells: immune cells that suppress inflammation by keeping the immune system in check. “Our immune system is sort of like a loaded gun, and as soon as there’s a microbe, it wants to fire,” says Mazmanian. Mice lacking regulatory T cells develop allergies or autoimmune diseases, and research suggests that some microbes can increase their abundance or boost their activity. Kenya Honda, an immunologist at the University of Tokyo, has been investigating this link.

Honda’s research focuses on bacteria in the *Clostridium* genus, many species of which live symbiotically in the intestines of mice and humans (although others, like *C. difficile*, are highly pathogenic). His team took mice that had been bred to be free of microbes, and inoculated them with a mixture of 46 different *Clostridium* strains. Sure enough, this was a catalyst for the production of regulatory T cells in the colon; inoculation with other types of bacteria had little or no such impact<sup>2</sup>. The team then used the same 46 *Clostridium* strains to boost the microbiota of standard laboratory mice, which typically already have *Clostridium* bacteria at a low level, and subjected them to tests that would ordinarily provoke an allergic response. They found that the *Clostridium*-boosted mice exhibited much more muted allergic responses than a control group, suggesting that microflora rich in *Clostridium* can provide at least partial protection against allergies.

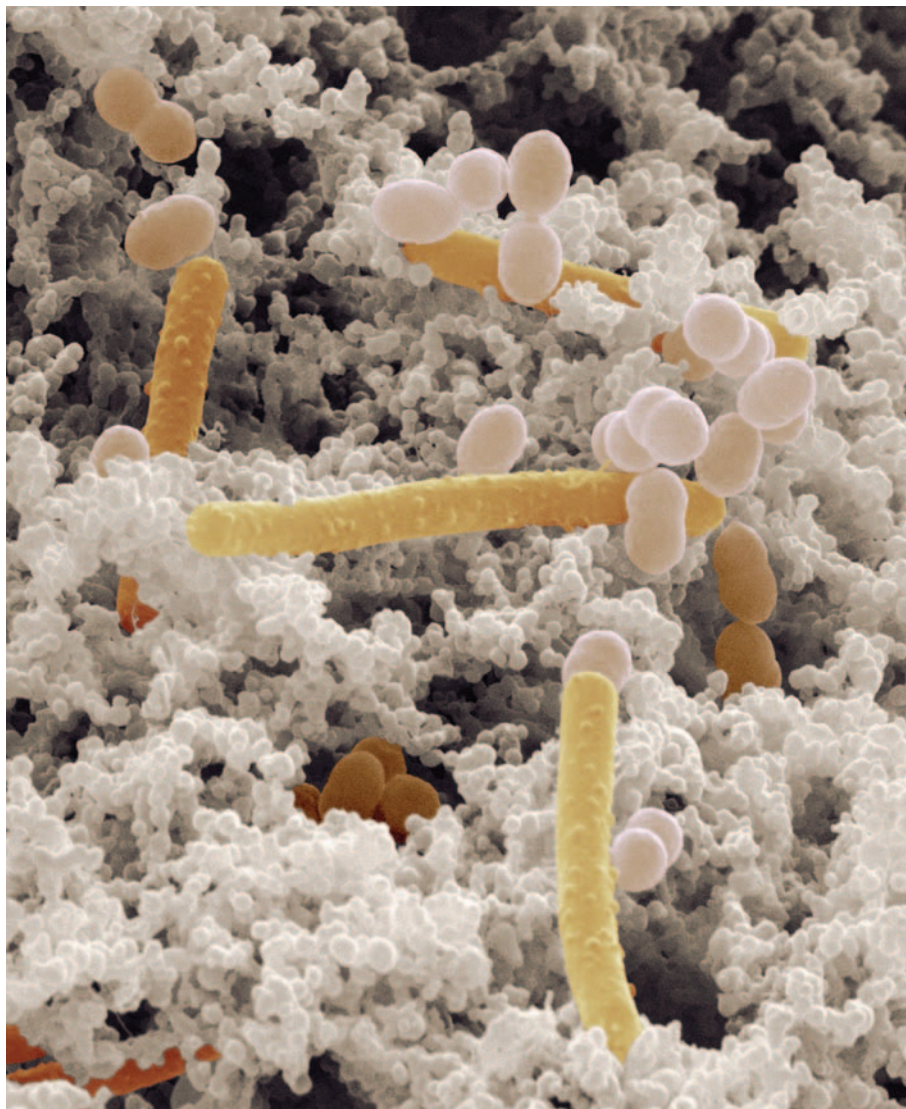
Another mouse study, yet to be published, reinforces Honda’s findings. A team led by Cathryn Nagler, an immunologist at the University of Chicago in Illinois, found that mice treated with antibiotics designed to eliminate *Clostridium* species had more food allergies than untreated mice. Nagler’s team also found fewer regulatory T cells in the lining of the colons of these mice. “One of our challenges now is to see how those regulatory cells get out of the colon to mediate protection against allergic disease,” Nagler says.

#### SEEKING OTHER SWITCHES

Mazmanian has focused his attention on an entirely different member of the gut microbiome, *Bacteroides fragilis*, which produces a molecule called polysaccharide A (PSA).

**Mice treated with antibiotics designed to eliminate *Clostridium* species had more food allergies than untreated mice.**

Mazmanian and his colleagues have already shown that PSA can prevent and treat inflammatory bowel disease and multiple sclerosis in mice, and speculates that it might work against allergies too. “It directly activates



Scanning electron micrograph of bacteria (coloured rods and spheres) among the milk solids in yoghurt.

those regulatory T cells,” he says, by signalling through Toll-like receptor 2 (TLR2), a protein found on T cells and other immune cells. Toll-like receptors bind to microbial molecules, and activation of this receptor typically ramps up immune activity. But Mazmanian found that PSA instead enhanced the function of immune-suppressing regulatory T cells<sup>3</sup>. Nagler’s work also implicates a Toll-like receptor, TLR4. In 2004, Mazmanian and her colleagues reported that a mutation in TLR4 made mice particularly susceptible to food allergies, and that administration of antibiotics to mice with normal TLR4s made them as susceptible to food allergies as their counterparts with defective receptors. TLRs appear to be one of the switches that commensals use to flip immune activity, although others are likely to exist, Mazmanian says. “There is indirect evidence that other commensal bacteria do not use TLRs to coordinate immune responses.”

#### BEYOND THE YOGHURT CURE

As bench scientists work to unravel exactly how commensals interact with the immune

system to curb allergies, clinicians and the food industry are searching for ways to tweak our microflora to prevent and treat allergic diseases. Yoghurt and other cultured food products labelled ‘probiotic’ contain bacteria said to be effective against a variety of ailments. More than 25 randomized clinical trials have examined the use of probiotics to treat or prevent allergies. Many of these studies have focused on strains of *Lactobacillus*, a genus of bacteria long used to manufacture cheese, yoghurt and other cultured foods.

In a 2001 study of newborns in Finland, one group of pregnant women with a family history of allergies were given a strain of *Lactobacillus casei* known as ‘GG’ during the last weeks of gestation, and their babies were given GG for the next six months. This appeared to reduce the children’s susceptibility to eczema, which often occurs in children who later develop asthma. At the age of two, the frequency of eczema in the probiotic group was half that of a placebo group. A follow-up study published in 2007 suggested that the beneficial effects

were still evident at age seven. However, a more recent study in Germany with a similar protocol failed to find any effect of *Lactobacillus* GG on childhood eczema<sup>4</sup>.

Why the contradictory results? Marko Kalliomäki, a paediatrician at Turku University Hospital and author of the Finnish study, notes that the studies used different methods for diagnosing eczema. Genetics may have played a role as well, he says. Michael Cabana, chief of paediatrics at the University of California, San Francisco, who is testing the effects of the same *Lactobacillus* strain on eczema in 270 infants, speculates that dietary differences could be relevant too. “If you have a diet that’s already high in fermented foods and probiotics, you might not see the effect of a probiotic supplement,” he says.

Another study has found that 6-month-old infants who received supplements of *Lactobacillus* GG had other beneficial bacteria too, and that the microbial communities in their guts appeared to be resistant to perturbations and the growth of pathogens. The probiotic appears to be “affecting the whole community structure,” Cabana says. He likens it to baseball. “Sometimes mid-season a team will add one player, and that one player just changes the whole dynamic, and they become a championship team,” he says.

Research looking at using probiotics as a treatment for allergies is equally mixed. A 2008 review by the Cochrane Collaboration, based in Oxford, UK, included 12 randomized controlled trials. It concluded that the studies, taken together, “do not suggest that probiotics are an effective treatment for eczema”.

It’s possible that probiotics researchers have simply not yet found the correct bacterial strain or combination of strains. Clinical trials tend to examine strains with good safety profiles, so those with the longest safety record, such as *Lactobacillus*, “are the ones that get studied more”, Cabana says. Honda and his colleagues are now working to isolate *Clostridium* from the human gut, and intend to test whether these strains induce a regulatory T-cell response in humans like that observed in mice. “And perhaps those *Clostridium* species can be applied to treat allergies,” Honda says.

#### FORTIFYING THE BENEFICIAL BACTERIA

Another approach to enriching the microbiota is nourishing bacteria in the gut with substances called prebiotics. One such prebiotic is inulin — a compound produced by plants that humans cannot digest. Inulin travels through the digestive tract to the colon. Once there, it provides food for bacteria, especially bifidobacteria — a genus found in breast-fed infants and also used as a probiotic.

Today’s prebiotics are what David Mills, a molecular biologist at the University of California, Davis, calls first generation and broad acting. As yet, he says, “there are no prebiotics that are tailored to enrich a specific population”.

## THE LUNG LINK

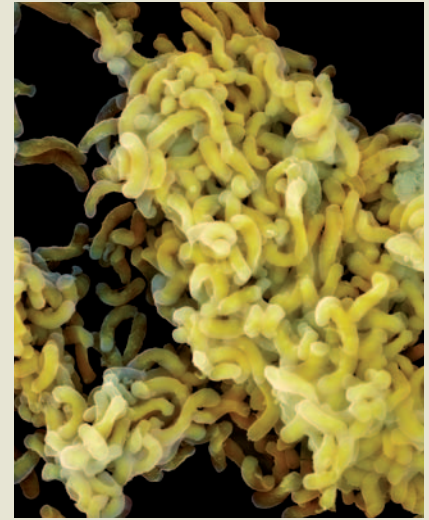
### Bacteria’s role in asthma

It’s not just microbes in the gut that shape our susceptibility to allergies. Bacteria in the lungs, once thought to be a sterile environment, might also play a role.

A study by Hans Bisgaard at the University of Copenhagen found that infants who had harmful bacteria in their lungs soon after birth were more likely to develop asthma than babies that didn’t have those strains. And in 2011, researchers at the University of California, San Francisco, led by Homer Boushey, reported that the lungs of asthmatic adults contained far more bacteria than the lungs of people without asthma. Furthermore, individuals with more severe cases of asthma had a greater diversity of bacteria than patients with less acute disease.

A stomach-dwelling bacterium long associated with ulcers and stomach cancer, *Helicobacter pylori*, has also been linked with asthma, but in this case the link appears to be beneficial. Studies by Martin Blaser, a microbiologist at New York University, show that children infected with *H. pylori* were 40% to 60% less likely to have asthma than children who weren’t infected.

A study, published in August 2011, supports Blaser’s finding<sup>5</sup>. Researchers in Switzerland and Germany infected 6-day-old mice with *H. pylori* and then attempted to induce an asthma-like disease by exposing them to allergens. The lungs of uninfected mice became inflamed, while mice infected with *H. pylori* didn’t experience any such allergic



*Helicobacter pylori* bacteria, coloured scanning electron micrograph

symptoms. Their lungs did become inflamed, however, after researchers gave them a dose of antibiotics to eradicate the bacteria. The researchers also noticed that regulatory T cells accumulated in the lungs of infected mice, and they speculate that these cells keep the allergic response in check. Anne Müller, a cancer researcher at the University of Zurich and author on the study, points out that regulatory T cells can travel from one mucosal surface, such as the stomach, to another, like the lung, and in this way “a stomach-dwelling bacterium can influence systemic immune responses”.

In theory, probiotics and prebiotics could be combined to increase the propensity of particular bacteria to take up residence in the gut and thrive. Huffnagle predicts that, some day, patients will go to the doctor and be prescribed a combination of probiotics and prebiotics designed according to their individual floral profile. But this generation of probiotics may not be bacteria you’d want to eat in a cup of yoghurt. “Some of the stuff they make smells nasty,” Huffnagle says. “They’ll be capsuled.”

Another approach could be to isolate and administer only the beneficial molecules produced by the microbes. Mazmanian, for example, speculates that “some day PSA could be a drug, just like insulin”.

There are many obstacles to overcome. Researchers don’t yet know what a healthy gut looks like, nor have they uncovered a profile for the ‘allergic’ gut. Although the evidence suggests that our microflora play a role in

the development of allergies, Artis points to a host of other factors. “It’s host genetics coupled with lifestyle coupled with potential effects of commensal bacteria, all operating simultaneously to influence disease susceptibility,” he says.

The complex origins of a disease may help explain why some people appear to respond to probiotics while others do not. Therapies that work for one person may not work for another, Huffnagle says. “Fixing the ‘broken’ microflora — that’s still a shot in the dark.” ■

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