outlook

The microbial ambassadors of the immune system

The gut microbiome can protect against food allergies — and its dysfunction can directly affect digestive and immune function. **By Michael Eisenstein**

By the time we take our first spoonful of solid food, there is already a sprawling welcoming committee of microbes in our digestive tracts. These microbiota help infants to convert those early bites into beneficial nutrients and metabolites. But they also have the potential to fuel an unhealthy immune response that can ultimately give rise to food allergies.

Many descriptions of the immune system fall back on simplistic military metaphors of an army poised to recognize and attack foreign invaders such as bacteria. But the truth is more complicated. Somewhat paradoxically, bacteria also seem to have a crucial role in training our immune system to distinguish true threats from otherwise harmless foreign molecules associated with our diet and environment. "Microbial exposures in early life really do shape the trajectory of our immune systems," says Supinda Bunyavanich, an allergist and immunologist at the Icahn School of Medicine at Mount Sinai Hospital in New York.

Lifestyle and environmental factors can disrupt these intestinal ecosystems in our earliest days of life, and possibly even in the womb. In some infants, the resulting microbial imbalance — known as dysbiosis — undermines the function of their digestive tract and their broader immunological health, creating conditions that lead to allergic disease.

The true prevalence of food allergies is not fully understood. This is in part because many studies have relied on self-reporting. In such cases, a true antibody-mediated allergic response is often conflated with non-allergic reactions such as food intolerance, which are instead typically rooted in digestive or metabolic dysfunction. Nevertheless, the number of people with food allergies is rising: in some populations, food allergy now affects as many as 10% of children.

Cathryn Nagler, an immunologist at the University of Chicago in Illinois, points to this ongoing increase as a sign that food allergy cannot be understood purely in terms of the interplay between dietary allergens and host immunity. "Some element of the environment is regulating that response," she says. "That's what has driven us to go further into understanding the microbiome." By homing in on the causes and consequences of dysbiosis, she and her colleagues hope to develop interventions that could help restore microbiome health and provide lasting relief.

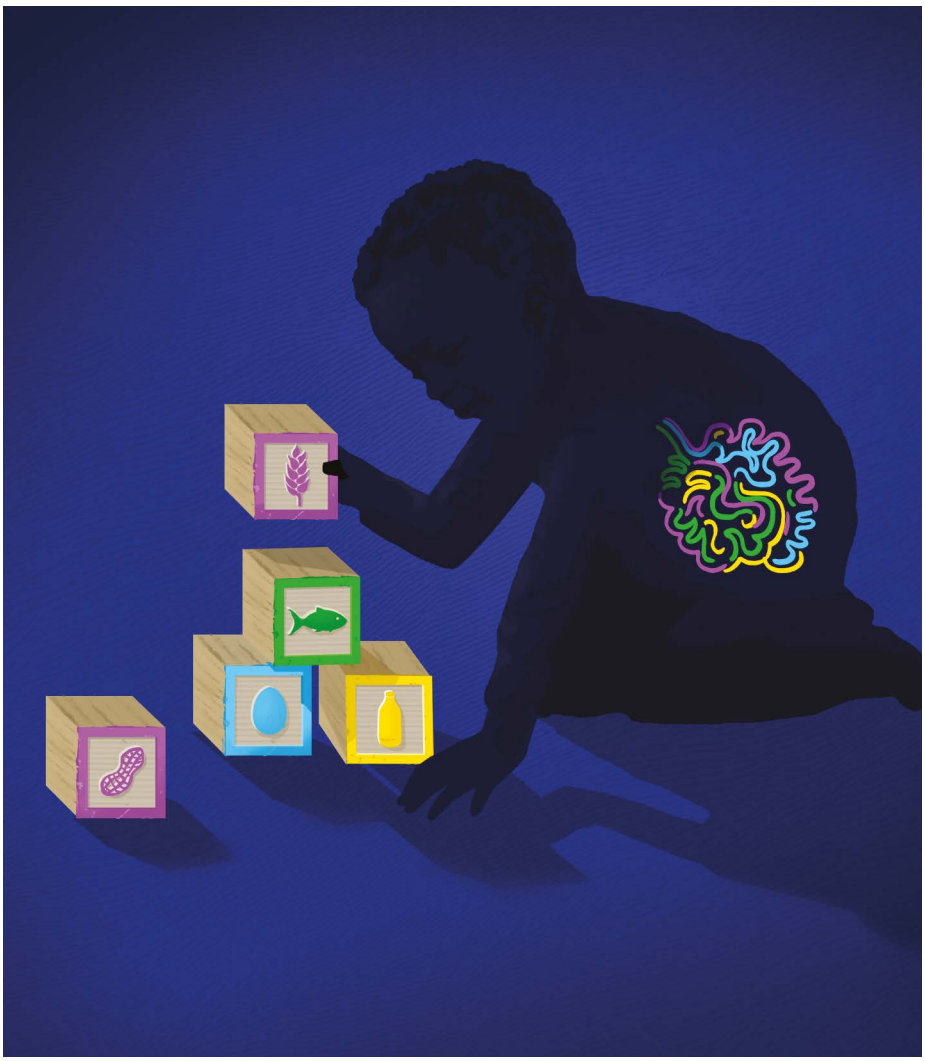

ILLUSTRATION BY SAM FALCONER

Hygiene hypothesis

The notion that early exposure to microbes can positively influence our immunological health dates back to at least 1989, when David Strachan, then an epidemiologist at the London School of Hygiene and Tropical Medicine, formulated the hygiene hypothesis[1]. On the basis of observational data, Strachan proposed that children who grow up in smaller, cleaner households are more susceptible to allergic disorders.

In the ensuing decades, the hygiene hypothesis has evolved into a microbiome-centred model in which early exposure to microbes

from our family and environment plays a crucial part in mitigating the risk of developing inflammatory conditions such as asthma, hay fever and eczema, as well as food allergies. These disorders share common roots in terms of immune dysfunction, and many children who develop a food allergy will experience other inflammatory conditions as they grow older. "Food allergy is kind of a canary in the coal mine in terms of immune dysregulation in the modern environment," says Peter Vuillermin, a paediatrician at Barwon Health and Deakin University in Geelong, Australia.

We assemble our gut microbiome early in life with organisms accumulated from our mothers, diets, homes and even pets. There seems to be a crucial window of development during which this community becomes firmly established, and this is also when the effects of dysbiosis can begin. For example, Bunyavanich and colleagues tracked the foundations of cow's-milk allergy to early infancy in a 2016 study, in which they monitored the diversity of microbiota species in a cohort of 226 children[2]. "We saw that gut microbial composition at ages three to six months had the biggest effects on food allergy outcomes," she says. Differences that appeared beyond this point had less impact. However, other studies have demonstrated that the microbiome continues to undergo meaningful reconfigurations throughout the first few years of life. "A lot of people talk about the first thousand days," says Nagler. "By the age of two or three, an individual has a relatively stable microbiome."

Antibiotics are also linked to the hygiene hypothesis. Their widespread use seems to be a major contributor to driving healthy microbiomes into dysbiosis. "American kids typically have six courses of antibiotics before the age of two," says Nagler. "Many of these are for viral ear infections, and so do a lot of damage to the developing microbiome to no purpose." She and her colleagues demonstrated the connection between antibiotics and food allergies in a seminal 2004 study[3], in which they induced peanut allergies in young rodents by killing off gut microbiota with broad-spectrum antibiotics. These experiments also revealed an immune cell receptor that seems to prevent the onset of allergies in response to microbiome-generated signals.

But the foundations for dysbiosis can be laid even at birth. Nagler notes that *Lactobacillus* species acquired during passage through the birth canal are typically among the first microbial populations to establish themselves in the infant gut. Children born by caesarean section (C-section) will not receive these bacteria, and this might affect the subsequent acquisition of species that rely on the lactate produced by lactobacilli as a food source. Although the data are limited, there is some evidence linking C-section deliveries with predisposition to food allergy, including one Swedish study that found a 21% increased risk relative to children delivered vaginally[4]. Newborns delivered by C-section might also be more prone to acquiring bacteria from the hospital environment, which can include pathogenic species. In addition, breastfeeding has an important role in building up the microbiome, and the transition to solid food at four to six months helps to establish a more adult-like microbiome. "There is a whole shift in the microbiome, with a bloom of clostridial species," explains Talal Chatila, an immunologist at Boston Children's Hospital in Massachusetts, adding that *Clostridium* bacteria seem to have a particularly prominent role in establishing immune tolerance to food antigens.

Misplaced microbes

Longitudinal studies of large cohorts of children have enabled researchers to monitor how changes in microbiome structure correlate with the onset of food allergies. Most of these studies have used 16S sequencing, which identifies the DNA signatures of microorganisms in a sample. The technique is generally not sensitive enough to identify every species and strain, but it can reveal subsets of microbes that contribute to immune dysfunction.

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Anita Kozyrskyj, an epidemiologist at the University of Alberta in Edmonton, Canada, has worked extensively with the Canadian Healthy Infant Longitudinal Development (CHILD) study, a multi-year effort to investigate factors associated with immunological disease in more than 3,500 children. In a 2015 study[5], her team performed 16S sequencing on fecal samples collected at the ages of 3 months and 1 year from 166 babies in the CHILD cohort. The sequencing revealed patterns in the microbiota of children who were later diagnosed with food allergy, such as a greater abundance of bacteria from the Enterobacteriaceae family relative to those from the Bacteroidaceae family. "We also found reduced species richness – which is a common measurement in microbiome studies – to be a risk factor," says Kozyrskyj.

Numerous studies have suggested that Clostridia bacteria in particular have an outsized role in modulating immune function. Researchers led by Christina West, a paediatric immunologist at Umeå University in Sweden, flagged multiple representatives of this microbial class in a 2019 study[6] that sought to link perturbations in the infant microbiome to a diagnosis of allergic disease later in childhood. "We could see that there was a consistent underrepresentation from infancy to school age of *Coprococcus*, which is a member of the Clostridia class, and temporary underrepresentation of another member of this class," says West. She notes that *Coprococcus* tends to reside in close proximity to the intestinal cells, suggesting that there might be direct cross-talk between these microbes and their host. West is now looking to dive deeper into the microbial correlates of allergy and the factors that shape them in a research programme called NorthPop. This massive longitudinal cohort will ultimately include 10,000 pregnant women and their families from the Västerbotten region of Sweden.

Another longitudinal research initiative, the Barwon Infant Study in Australia, has uncovered intriguing evidence that the microbiota in a pregnant woman's gut influence the onset of food allergies in children. After analysing fecal samples from 316 women and their babies[7], the researchers found that the presence of the microbial species *Prevotella copri* in the maternal gut strongly correlated with the absence of food allergies in infants by the time they reached one year of age. "This association does not appear to be mediated by the baby's carriage of *P. copri* postnatally," adds Vuillermin, lead author on the study. This suggests that *P. copri* might instead shape fetal immune development, perhaps by secreting beneficial metabolites that cross the placenta, although Vuillermin and his colleagues are still working to clarify the nature of the microbe's influence.

Metabolic mediators

A number of research groups are working to identify the molecular messages that pass between gut microbes and their hosts to understand how they influence allergic disease. Many of these signals seem to be closely linked to compounds that bacteria generate as they metabolize food in our intestine. Of particular interest are short-chain fatty acids (SCFAs), a class of molecules produced by bacterial digestion of dietary fibre. Butyrate is the most well-studied SCFA in terms of its beneficial effects, but researchers have also identified roles for other members of this chemical family, including acetate and formate.

SCFAs and other bacterial metabolites are thought to shape the risk of allergic disease through two distinct but interconnected

mechanisms. The first involves their role in training the immune system to recognize allergens from foods such as peanuts and eggs as safe and not worthy of an aggressive inflammatory response. "There are populations of regulatory T cells that normally enable immune tolerance to gut content, including food and bacteria," says Chatila. "And these cells are very dependent on signals from the bacteria." A dysbiotic gut is thought to be insufficiently capable of producing SCFAs and other compounds that act as signals for regulatory T cells, resulting in a lack of immune tolerance towards dietary allergens.

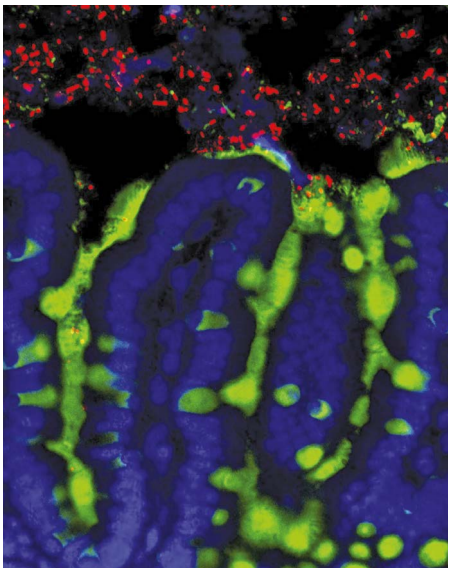
The other mechanism involves allergens breaking out of the gut. Perturbations in the microbiota can undermine the structural integrity of the intestinal lining, turning what should be a tight barrier into a porous one. "Food-allergic infants and children have a leaky gut, so there is increased antigen and allergen transfer," says West. The interior of our digestive tract is normally shielded from immune surveillance, but if this barrier becomes leaky, food-derived proteins can seep through and trigger a hostile response. According to Nagler, several of the foods that provoke the most common childhood allergies, such as peanuts and cow's milk, contain durable proteins that are likely to pass intact through the compromised gut barrier and trigger an immune response. She and her colleagues have detected intact peanut protein in the blood of mice that had been treated with antibiotics[8]. However, this was not the case in antibiotic-treated mice that had also been given Clostridia bacteria, she says — suggesting that this class of microbes can help to mitigate allergy risk.

Many of the details of the microbiome–host relationship have been teased out through studies with germ-free mice. These animals are reared in sterile conditions and thus lack a gut microbiome, but can be colonized with hand-picked subsets of microbial species to study how they affect gut and immune function. Chatila's group has used such animals to identify a signalling pathway that specifically triggers the activation of regulatory T cells in response to microbial signals.

In 2019, his team was able to home in on a subset of Clostridia species from the human microbiome that can act on this immune pathway to quell food allergy when transplanted into germ-free mice[9]. "You can cure food allergy in these mice, or prevent it from happening," says Chatila.

Hacking the microbial network

For well over a decade, researchers have been exploring the idea that transplanting one or more hand-picked microbial species might be sufficient to reverse a dysbiotic human gut. West's experiences from a handful of clinical studies with such probiotic treatments have generally been disappointing, however. Although the transplanted microbes established themselves successfully in infants, the bacteria tended to die off in later childhood. "When these children were at school age, there were no differences in terms of gut microbiota, and no differences in terms of immune responses," she says. This suggests that transplanted microbes might require additional support to successfully take hold in an established microbiome community.



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Bacteria (red) in a mouse small intestine.

One possible solution would be a 'synbiotic' formulation, in which potentially therapeutic species are packaged with their favourite food sources — for example, certain complex sugars found in breast milk. The idea is that this could give the microbes an advantage in establishing a stable population. In a 2019 study, Nagler and her colleagues zeroed in on a Clostridia species, *Anaerostipes caccae*, which could single-handedly reverse cow's milk allergy in a germ-free mouse model[10]. She is exploring the potential of synbiotic formulations of this species through her start-up company, Chicago-based ClostraBio, although her team has yet to find the ideal food source for the bacterium. "You should be able to repopulate the gut with that bug over a relatively short period of time," she says. Many of *A. caccae*'s effects on the gut are facilitated through its production of butyrate, and ClostraBio is exploring the potential of delivering butyrate directly to patients through a controlled release formulation developed by co-founder Jeffrey Hubbell.

Another option is to reboot the system with a fecal microbiome transplant, using a donor's healthy microbiota to replace or repair a dysbiotic gut community. Studies in germ-free mice have demonstrated the feasibility of restoring healthy immune function in this way, and this approach is already used in the clinic to repair the microbiome damage in patients infected by the pathogenic bacterium *Clostridium difficile*.

But performing such procedures in young children with still-developing immune systems raises additional safety concerns. "There are so many unknowns," says West. "The downside of this could be that you would transfer potentially pathogenic bacteria." Her team is now exploring the feasibility of this approach in a clinical trial in which infants delivered by C-section will be treated with a mixture of their mother's vaginal and gut microbes in an effort to prevent the potential onset of allergic disease.

One of the fundamental challenges in developing therapies is that the diversity of dysbiotic states remains poorly understood. Indeed, some data suggest that the roads to allergy differ for various foods. "My perspective is that there probably are differences depending on the subtype of food allergy," says Bunyavanich.

But despite these differences, if researchers can ultimately identify a core set of microbial species and functions that promote the development of a stable and immune-protected gut environment, it should be possible to devise broadly protective microbiome-oriented strategies for reversing the onset of allergy. This is particularly appealing given the modest success of currently available interventions such as oral immunotherapy, in which children must be routinely inoculated with the allergy-inducing food for years on end. "These therapies are time-consuming, they have side effects, and you have to reproduce them for different allergens," says Chatila. "There is an enormous enticement to come up with therapies that would durably reset tolerance not only for one allergen, but for potentially any allergen."

Michael Eisenstein is a science writer in Philadelphia.

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