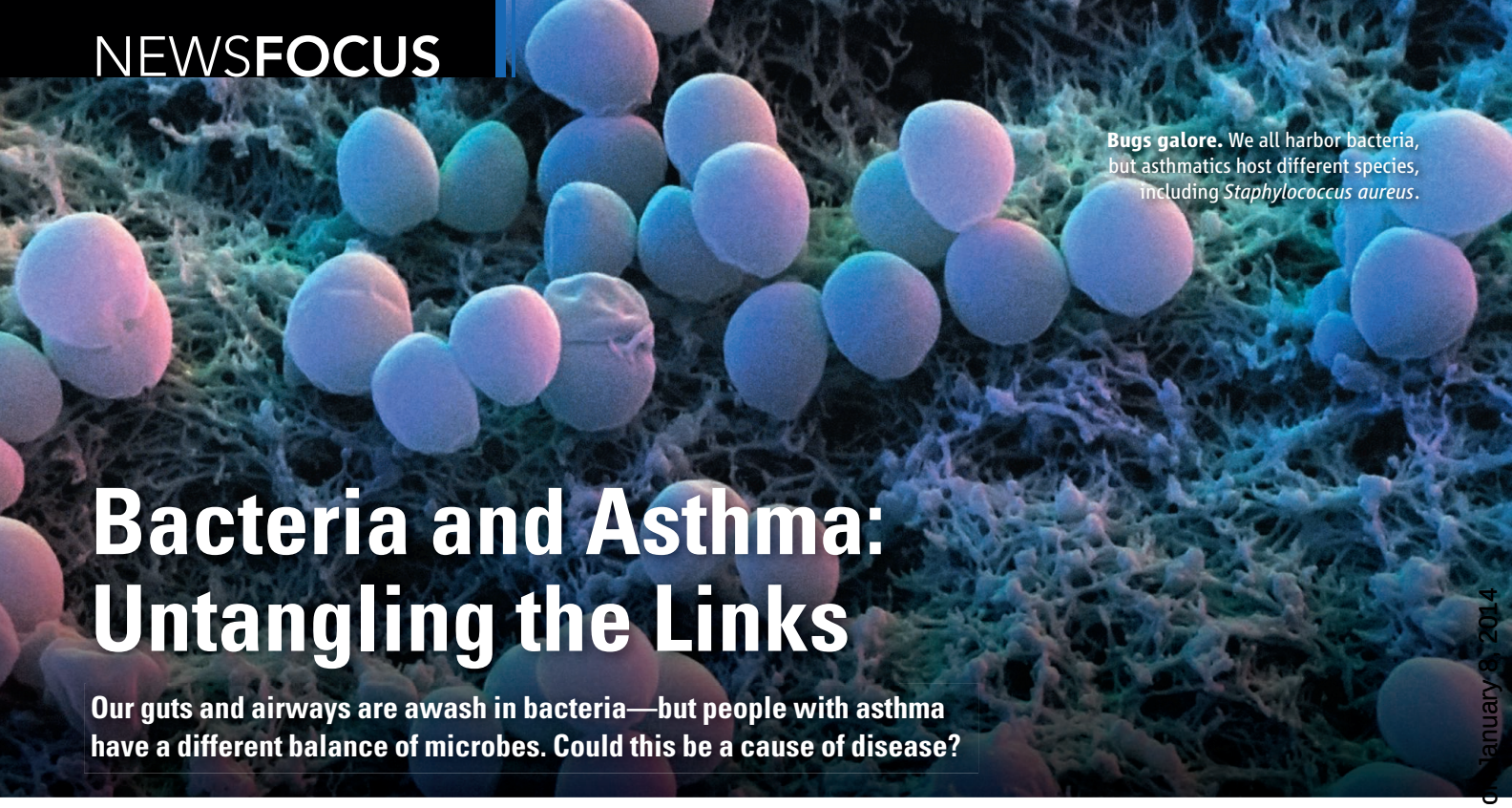


Bugs galore. We all harbor bacteria, but asthmatics host different species, including *Staphylococcus aureus*.

Bacteria and Asthma: Untangling the Links

Our guts and airways are awash in bacteria—but people with asthma have a different balance of microbes. Could this be a cause of disease?

SIX YEARS AGO, GARY HUFFNAGLE, AN immunologist at the University of Michigan, Ann Arbor, conducted an experiment that reflects what happens to many of us early in life. He exposed mice to a triple whammy: yeast in their intestines, mold spores up their noses that migrated down the airways, and an antibiotic drug. The animals began showing signs of asthma; blood tests revealed disruption of their immune systems.

"They developed some fairly wicked allergic disease in the lung," says Huffnagle. The mold and yeast alone didn't make much difference. "The bottom line is, those animals are perfectly healthy until we hit them with an antibiotic."

Huffnagle's study, published in 2004 in *Infection and Immunity*, was one of the first pieces in a dizzying new jigsaw puzzle of asthma, whose causes remain elusive even as the number of cases is soaring. Researchers have some striking clues: For example, children on farms are much less likely to get the lung disease. "A rich microbial environment in childhood is somehow protective," says William Cookson, a respiratory physician and geneticist at Imperial College London. Cookson and others are now moving from the outside environment to deep in the body, exploring whether bacteria there might influence the onset of asthma.

As odd as this might sound, there's mounting evidence that bacteria matter. Babies born via cesarean section, who experience a more sterile entry into the world than those born vaginally, are more likely to get asthma. So are young children treated with many courses of antibiotics. Along with animal studies, these observations suggest that the balance of bacteria and other microbes help guide immune development—and that when the balance is disrupted, disease may follow.

The picture can be dishearteningly complicated. Thousands of species of bacteria have constructed virtual cities inside us, along with fungi and viruses—a world called the microbiome. And it's not so much the presence or absence of bacteria, or even certain species, that matter, but rather the shape of the whole community. All of us play host to bacterial residents. But children who develop asthma, researchers are learning, are home to different bacteria—and sometimes a less diverse mix—than those who stay healthy. "It's really coming down to the bacterial community structure, who's there, and in what numbers, and where," Huffnagle says. Cataloging these inhabitants is a new frontier.

Sterile birth. C-sections, which expose babies to fewer bacteria than vaginal births, are linked to a higher risk of asthma.

Lungs and guts

For many years Hans Bisgaard, a pediatrician at the University of Copenhagen, was puzzled by a classic feature of asthma: Very young children with the disease have abundant neutrophils in their lungs, white blood cells that generally appear when the body is fighting infection. Given that asthma isn't considered an infectious disease, this reaction seemed out of place.

Beginning in 2000, Bisgaard had a chance to assuage his curiosity. He and his colleagues were recruiting about 400 pregnant women with asthma to track the onset of disease in their offspring. They took throat swabs from the babies when they were 4 weeks old and looked for certain pathogenic bacteria. To their great surprise, about a fifth harbored these microbes in their airways, including strains of influenza and pneumonia.

The newborns weren't sick and so were left untreated. Five years later, 33% of those who had had pathogenic bacteria early on had asthma—compared with 10% of those without the bacteria. The work was published 3 years ago in *The*

CREDITS (TOP TO BOTTOM): DAVID SCHARF/SCIENCE FACTION/CORBIS, DRPH/CDC; ISTOCKPHOTO

Published by AAAS

New England Journal of Medicine. "It's redefined our course of research," says Bisgaard. His team has since launched a second cohort study, this one of 800 pregnant women, most of them healthy, and has found the same prevalence of airway bacteria in newborns.

The work also upended how researchers think about lung biology. "If you read a medical textbook even now, it will say the lungs and the airways are sterile; there aren't any bacteria down there," says Cookson. He became certain that the conventional view was incorrect when he and an Imperial College colleague, geneticist Miriam Moffatt, conducted their own variation of Bisgaard's study in babies. They had at their disposal advanced gene-sequencing techniques that allow for a much more comprehensive census of bacteria flourishing in the lungs. In January, the two and their colleagues wrote in *PLoS ONE* that they'd sequenced more than 5000 different species in 43 people, including some with asthma and others who were healthy.

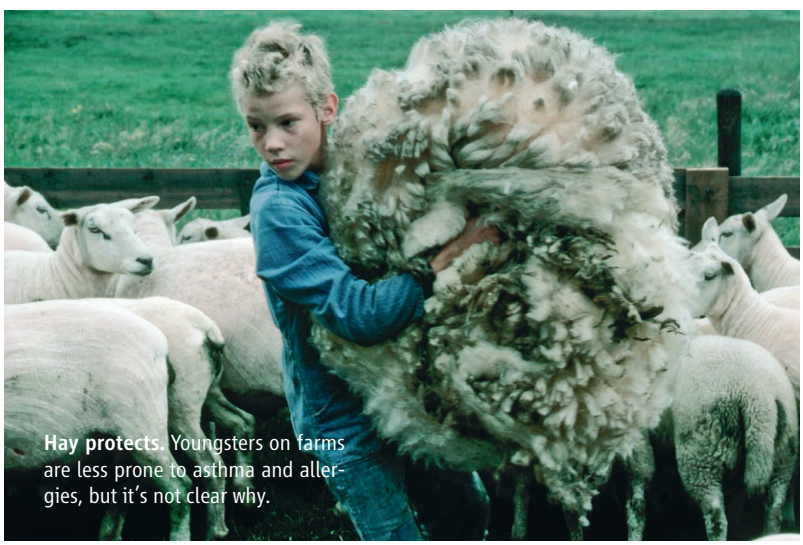
The bacterial balance was quite different in the asthmatics: Affected children had more proteobacteria, which include pathogens like influenza. A different class—bacteria called bacteroidetes, found in soil, seawater, the gut, and skin—were more common in the nonasthmatics. Cookson and Moffatt are now collaborating with Bisgaard to conduct gene sequencing of lung bacteria in Bisgaard's Copenhagen cohort.

Similar discoveries may help explain the association between birth by C-section or abundant antibiotic use and increased risk of asthma in childhood. In Dutch research published in 2008, 8-year-olds born by C-section had nearly double the risk. Both C-sections and antibiotics modify the flora of gut and possibly lung bacteria, in some cases for many years. "The microbiota of children born through C-section is different; it's less diverse," and the same is true for antibiotic use, with fewer species present, says Anita Kozyrskyj, an epidemiologist at the University of Manitoba in Winnipeg, Canada.

And just as Cookson found different bacteria in the lungs of asthmatics, there's growing evidence that this is true in the gut, too, says Fernando Martinez, a pediatric pulmonologist at the University of Arizona, Tucson.

Researchers are probing the connection with animal work and following thousands of children from birth. A Canadian effort called CHILD is recruiting 5000 pregnant women and will track their children for at least 5 years. "We have lots of poop frozen away"—including the newborn's first, right after birth—"dust from the babies' beds, nasal swabs, [a] massive library of this information that we'll be able to piece together," says Stuart Turvey, a pediatric immunologist at the University of British Columbia in Vancouver, one of CHILD's leaders.

So far, the evidence linking asthma and bacteria are associations, not proof that an imbalance of bacteria causes the disease. The big question, says Martinez, is, "Do asthmatics have an immune system that makes them be colonized by different things? … Or is it because they were colonized by different things that caused them to have asthma?"



Hay protects. Youngsters on farms are less prone to asthma and allergies, but it's not clear why.

CREDIT: GREHAN FARRELL/CORBIS

From the inside out

Even less is known about what might be modifying lung and gut microbial communities and why children exposed to the same factors respond differently. The pattern isn't clear-cut: Many children born vaginally, or who never got antibiotics, still develop asthma, just as many born by C-section or given antibiotics do not.

One piece of the puzzle is likely to be genetics: Some babies may be more prone to colonization by certain microbes, or to developing asthma once that colonization occurs. Another piece is the environment. For years it's been known that children raised on farms are less likely to develop asthma and allergies than others. This phenomenon is often referred to as the "hygiene hypothesis," the idea that the relatively germ-free lifestyle most of us now lead can disrupt the development of the immune system.

In the countryside of five European countries, Erika von Mutius, a pediatrician and epidemiologist at Munich University Children's Hospital in Germany, is sifting for clues. She and others are planning to sequence stool samples and throat swabs from more than 1100 children—half on farms and half in nonfarm rural settings. They're also collecting samples of milk (farm milk is often unprocessed) and the components of dust for limited gene sequencing. "A lot of this is inhaled," and because we're talking about young children, "a lot of this is also probably ingested," says von Mutius.

Although researchers assume that a child's microbiome is affected by the environment, they don't know this for sure. And proving definitively that bacteria help cause asthma is remarkably difficult. "The only proof lies in a randomized controlled trial, where you somehow manipulate exposure" and see who gets sick, says Bisgaard.

Researchers are experimenting with this approach in the gut. Probiotics, microorganisms like *Lactobacillus* found in yogurt, could in theory be helpful, but small trials testing whether they prevent allergic disease haven't been definitive. In 2006, the University of California, San Francisco, began recruiting about 200 babies who have at least one parent with asthma. Half receive a probiotic and half get a placebo, and the researchers are focusing on early markers linked to asthma, like eczema and wheezing. In January, they reported that 6 months of probiotics in infancy did alter the balance of microbes in the babies' guts, but final results are several years off.

However these studies turn out, there's no question that asthma has diverse triggers. Huffnagle's mice needed a series of insults—gut yeast, antibiotics, and mold—to cause an explosion of symptoms. If the bacterial communities living in children wind up high on the list of risk factors, that may help solve one of the biggest mysteries of all in asthma: why the number of cases has climbed so high, and how it might be pulled back down.

–JENNIFER COUZIN-FRANKEL