

Our Body the Ecosystem

Even a healthy human body is teeming with bacteria. Studying the subtle interplay between man and microbe could help doctors understand diseases that affect millions. It could also one day help eczema patients from ever itching again

BY VIRGINIA HUGHES
PHOTOGRAPHS BY HOWARD SCHATZ

WHEN JAKE HARVEY VISITS the clinical center at the National Institutes of Health in Bethesda, Maryland, he is usually dirty, itchy and wheezing—not the happiest state of affairs for a 14-year-old boy. But his doctors require that for 24 hours prior to each visit, he refrain from bathing, or using the inhaler that soothes his asthma, or applying the ointment that softens his eczema. In order to study his illness, they need him to be in as close to his natural state as possible.

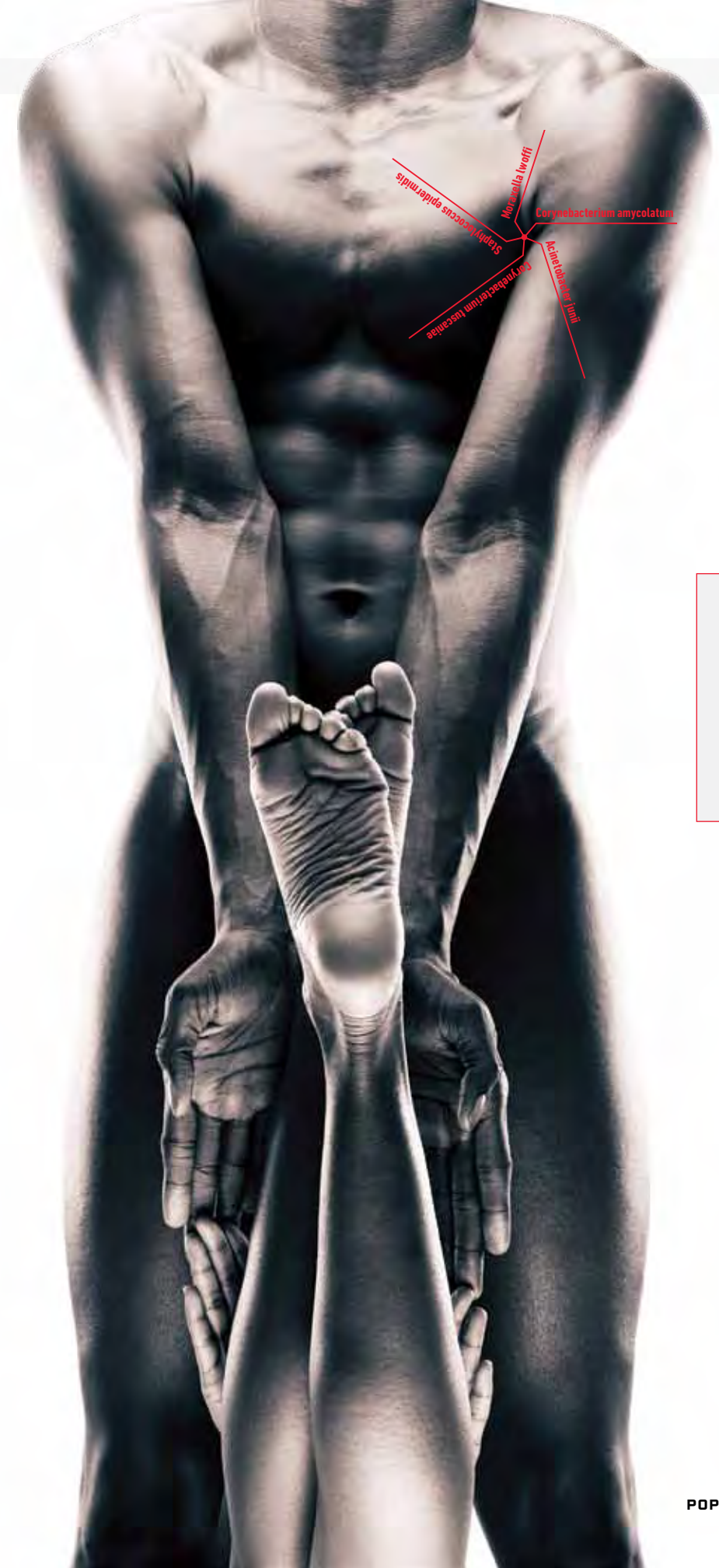
Jake's discomfort could lead to better treatments for the millions who have eczema—a disorder marked by dry red rashes in the creases of elbows, behind knees and on the back of necks—as well as an array of other allergic reactions. By understanding eczema in a new way, as the product of a delicate interaction between the

immune system and the legion of bacteria that live on the skin, one group of scientists hopes to better understand what triggers it and why the number of diagnosed eczema cases in developed countries has dramatically increased over the past few decades.

These researchers, led by Heidi Kong, a dermatologist at the Center

for Cancer Research at the National Cancer Institute and Julie Segre, a geneticist at the NIH, are just one part of the five-year, \$173-million Human Microbiome Project (HMP), an effort to characterize the thousands of species of microbes that live on or in us. So far, Jake has made half a dozen trips to Bethesda, 60 miles each way, to donate a few skin cells to the project.

Jake has been struggling with eczema since he was a few months old. The rash never stops itching, and when he scratches, it bleeds and scabs and gets even itchier. His clothes stick to the sores. He has tried many treatments, including petroleum jelly, topical steroids, antibiotics, and also dairy-free, gluten-free and probiotic diets. None of them has worked very well.



SKIN MAP
Dermatologists divide skin into three major types: oily, moist and dry. Each region harbors distinct classes of bacteria.

When he was younger, he went to school with bandages on the tips of his fingers and slept with socks over his hands. In bed, he still sometimes lies on his back with both legs sticking straight up so they're easier to scratch. "I've never really gotten a full night's sleep," he says.

As many as 30 percent of all children develop eczema, and no one knows what mix of genetic and environmental factors sets it off. The disease runs in families, yet Jake's twin sister, Becca, has perfect skin. For about 60 percent of children with the disease, it goes away by early adolescence. The others frequently deal with outbreaks for life.

Whether the rash disappears or not, nearly one third of children with eczema go on, like Jake, to develop asthma and hay fever. Asthma and hay fever also involve inflammation, but very little is known about what quirk of the immune system links them all together. "We've been to pediatricians, allergists, dermatologists for years," says Jake's mother, Debbie, "and nobody can figure him out."

THE AVERAGE HUMAN body is made up of trillions of cells. The average human body also houses about *10 times* that number of bacterial cells. Scientists have been curious about our bacterial cohabitants since 1683, when Antonie van Leeuwenhoek, using a microscope he had built himself, examined his own dental plaque only to discover "little living animacules, very prettily a-moving." But it has been only within the past few decades that scientists have begun to understand just how many varieties of bacteria live in or on our bodies. And now they increasingly suspect that many diseases are caused not by individual bacteria, but by the delicate interplay between multiple bacterial species and the human host.

In the Human Microbiome

Project, researchers plan to characterize the vast numbers of bacteria, fungi, protozoa and viruses in our body by sequencing their genes. That won't be easy. In the past, researchers had to grow each species outside the body before they could identify it, a process that required intense research to determine optimum growing conditions. Only the hardiest and most numerous bacterial species—for example, *Staphylococcus aureus* and *Streptococcus pyogenes*, which can cause life-threatening infections—have been thoroughly studied in the laboratory. Now, advances in DNA sequencing—the very same that made it possible for the Human Genome Project to decode the three billion base pairs of our own genome quickly—have provided the technology to make a comprehensive Human Microbiome Project possible.

"What we want to understand first," says Human Microbiome Project coordinator Lita Proctor, "is what's considered the norm. What does a typical healthy human have?" HMP researchers are building a reference database of the genetic fingerprints of about 3,000 different bacterial species. Scientists are also thoroughly characterizing the makeup of microbial communities found on half a dozen body sites—including the gut, the mouth, the skin and the groin—of 300 normal people. The next step is to compare those results with what researchers find in patients with specific medical conditions such as eczema, Crohn's disease and ulcerative colitis.

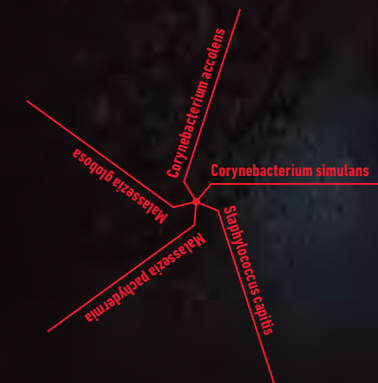
The skin samples from Jake and other children with eczema will help Segre and Kong determine whether changing profiles of skin flora, and its interaction with the human immune system, are involved in the rising rates of the disease. Some 34.1 million Americans suffer from asthma, and up to 50 million have seasonal allergies.

"In the last three decades, all of these allergic disorders—asthma, eczema, hay fever—they've all tripled," Segre says. With that sort of a time frame, the culprit can't be simply changes in our own genome. "So it must be something about the gene-environment interaction. And I now believe that that's modulated by the body's bacteria."

OILY

The scalp, upper back and crease of the nose all have an abundance of oil-producing sebaceous glands. Oily regions tend to have less bacterial diversity than other regions.

THE FIRST PART of the exam is like any other doctor's visit, Jake says. Sitting on a stool next to him, Kong asks a series of questions. *Which medications are you currently taking? Does your school use antibacterial soaps? How bad are your allergies this month?* On a diagram of a body, she notes all the spots where Jake's rash has cropped



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up that day. Then she pulls on a pair of blue examination gloves and turns to a tray containing a row of sterile swabs and scalpels. The swabbing comes first. Jake stretches out his right arm, palm up, and Kong firmly rubs a wet foam swab in a circular motion in the crook of his elbow; the sterile water solution stings when it hits his open sores. Kong places the tip of the swab into a plastic tube filled with the same liquid and places the tube in a bucket of dry ice. Then comes the scalpel. Kong moistens the blade and gently scrapes some skin from a spot next to the area she swabbed. She wipes the flakes from the blade with another swab and drops the tip into another plastic tube. Kong repeats the procedure on each elbow, each inner forearm, the back of each knee, and finally a swab in one of his nostrils. Once the sampling is over, she and her colleagues take pictures of Jake's rash. Then they check his vital signs.

Jake doesn't know much about what happens to his skin cells once they disappear into the lab. For the most part, he comes to the clinic, which he found because he was enrolled in an NIH study on asthma, for Kong's helpful advice about his eczema. (She recommended that he take diluted bleach baths during bad flares, which has given him some relief.) But he says he's proud to be part of the effort to understand this disease, in case his future children end up with the same troubles.

Jake's skin samples, like those from the more than 20 other children participating in the study, wind up in a nearby laboratory complex, where they are stored in a large freezer set at -112°F . The inside of the door is lined with thick white snow. Clay Deming, a biologist at the lab, explained to me how he prepares each sample. He selects a tube and defrosts it; adds lysozyme, an enzyme that breaks down bacterial cell walls; and shakes the concoction using a vortex mixer. This releases DNA

from the bacterial cells so that it floats freely in the liquid. He then pours the solution over a gel filter, designed so that just bacterial DNA fragments will stick to it.

With the DNA fragments thus separated, Deming can use a technique called polymerase chain reaction to make millions of copies of one particular bacterial gene: 16S. All bacteria carry the gene, but its DNA sequence varies from species to species. After amplifying the DNA fragments, Deming is left with a new set of tubes, each holding the 16S pieces harvested from a particular area of the participant's skin.

He puts the tubes on ice in order to FedEx them to a building five miles north, the NIH Intramural Sequencing Center. There, machines read the precise sequence of 1,500-odd letters in each 16S fragment, giving the chemical signature of each species. Finally, technicians upload the sequence data to an internal network, ready to be mined by Segre and Kong.

THE SKIN IS AN ECOSYSTEM.

Like any other ecosystem, it harbors permanent residents and also migrant species that flock to a few hotspots during certain seasons. Those fluctuations powerfully influence how the skin works. *Staphylococcus epidermidis*, for example, may help educate the skin's immune system, training it to recognize particular molecules so that it can better respond to an attack by harmful species. *S. epidermidis* churns out proteins that prevent unwanted invaders from adhering to skin. So it makes sense that disrupting these complex microbial interactions could lead to skin problems.

With the eczema study, Segre says she hopes to find patterns in the microbiome that could predict the onset of an eczema flare in a particular child, or even help doctors choose a far more effective treatment; for some patients, bleach

baths might help, whereas for others, a round of anti-inflammatory steroids might be the best choice.

Kong and Segre are not the only scientists looking into the connection between the microbiome and inflammatory skin conditions. Martin Blaser, a microbiologist and physician at New York University, thinks the microbiome also plays a role in psoriasis. Some 25 years ago, Blaser's father had told him that his psoriasis—scaly red-and-white patches on the skin—seemed to get better after he started taking allopurinol, a medicine that treats gout. Blaser, who has mild psoriasis himself, took notice. He knew that because allopurinol interferes with the synthesis of DNA, it kills bacteria or makes it very difficult for those bacteria to grow.

His father's experience spurred Blaser to look deeper into the connection between allopurinol and microbes. He discovered that a few decades earlier, researchers had run clinical trials on allopurinol as a treatment for psoriasis. The results were inconclusive, but Blaser thought that the medicine might work on a particular subgroup of psoriasis patients.

In 2002, he finally secured funding to study the microbiomes of psoriasis patients. For the project, Blaser performed a general census of bacteria living on the inner forearms of six healthy people. Later his group did the same analysis on skin from six people with psoriasis, and they found some striking differences. The six psoriasis patients had considerably more bacteria from the *Firmicutes* phylum than people without psoriasis. What's more, the most common class of bacteria in healthy skin, *Actinobacteria*, was significantly underrepresented in the lesions.

"Those results, which were important at the time, now are kind of laughable because they're on such a small scale," Blaser



MOIST
Inside the nose, between the fingers and toes, and the heel—these areas of skin and folds contain many sweat glands. Eczema flares are sometimes found in moist skin areas.

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says. Now, with funding from the Human Microbiome Project, his team is looking in much greater detail at how the overall microbial numbers, as well as changes in the species distribution, vary in patients with psoriasis.

In May 2009, Kong, Segre and their colleagues published the first comprehensive catalog of skin microbes, based on samples taken from 20 different body parts—from the oily crease outside the nose to the moist spots between the fingers—of 10 healthy people. By sequencing hundreds of the 16S genes in each sample, the researchers found that our epidermal ecosystem is much more diverse than anybody thought, with discrete bacterial populations ranging in size from 15 species behind the ear to 44 on the forearm. The team also looked at whether the microbiome is consistent across individuals; that is, do some individuals always have a certain set of bacterial species, no matter what part of the body those bacteria live

in? The researchers learned that, no, the bacteria are finely tailored to specific locations on the body, rather than to individual humans. For example, the bacterial communities under your arm are more similar to those under someone else's arm than to those behind your knee.

JAKE IS ALLERGIC to many things—mold, peanut butter, dogs, cats—and his asthma is severe. In 2009 he almost died because he couldn't breathe. "The scary thing was, within seconds, he was in respiratory and cardiac arrest," his mother recalls. "No warning. No lip swelling or dizziness or mouth burning. He just went down."

Eczema's strong link to allergies suggests that the immune system is key to the development of the disease, and researchers have focused on inflammation for decades. So how does the skin microbiome fit into this picture? In 2006, a coalition of researchers from the U.S., France, Ireland and the U.K. discovered that up to

half of people with eczema have mutations in flaggrin, a protein in the skin barrier, or top layers of the skin. Now some researchers suspect that this flaw in the skin barrier allows entry to particles that trigger the immune response and creates an ecological niche for a different set of microbes.

To uncover any link between the skin barrier and microbes, Kong and Segre are sampling Jake and other children with eczema at three points: during a normal, or baseline, period; during a flare; and two weeks after treatment. In a preliminary analysis of data from 10 patients, Kong and Segre have confirmed older studies showing that there are huge amounts of *S. aureus* on the skin during a flare. What wasn't known before, however, is that *S. aureus* crowds out the other bacterial species during a flare. Kong and Segre are trying to figure out how treating these patients changes their bacterial diversity and leads to better individual results.

DRY

The forearms and buttocks have a relative paucity of sweat and sebaceous glands. Bacteria from dry skin sites are typically the hardest to grow in culture.

Staphylococcus epidermidis
Staphylococcus hominis

Hospitals are already swabbing people's noses to screen for drug-resistant bacteria. "You get results in less than an hour," Segre says.

Segre doesn't know whether bacteria associated with eczema are the cause of the disease or simply a consequence of living with it. To find out, she plans to perform a metagenomic analysis of the samples. During a metagenomic analysis, scientists compare thousands of genes present in a particular species's DNA. By looking at the biological function of the genes—what kinds of proteins they make and what kinds of biological pathways those proteins are involved in—the scientists can make educated guesses about the role of each species, and how different species may work with one

another and with our own genome. "We all believe there's an interdependency among these organisms. They're highly dependent on their neighbors for their survival," says Claire Fraser-Liggett, director of the Institute for Genome Sciences at the University of Maryland. Metagenomics, however, is immensely complicated. Researchers know little about how the millions of microbial genes might work together, and it's difficult to sort out which patterns are signatures of disease versus part of normal variation between people. "There's no way to overemphasize the analytical challenges," Fraser-

Liggett says. "It's something that everybody is struggling with."

Early results from Segre's study indicate that researchers might not have to decode the entire microbiome to better treat children's skin diseases. She says she envisions a day in the not-too-distant future when a dermatologist, during an office visit, will drop a skin sample into a machine that spits out a signature of the microbiome.

If she finds certain microbial profiles that predict the onset of an eczema flare, for example, doctors could use that data as a guide for action. They might tell the patient to take a few extra bleach baths that week, or to skip football practice. Researchers might even be able to create "probiotic" concoctions to replace the bacterial species that patients lack during a flare, Segre says. Hospitals are already routinely swabbing people's noses to screen for drug-resistant bacteria. "You get that result in less than an hour," Segre says. If the screen turns up MRSA, for example, doctors can prescribe an antibiotic known to be effective against the species.

These potential applications are many years off, and Segre's initial studies probably won't have much affect on Jake's eczema. Still, the Harveys are happy to be moving the field forward. "It's probably going to be a while before Jake's helped," Debbie says. "But in the future, if someone else can avoid sitting up all night scratching their legs, that would be great."

Virginia Hughes is a writer living in Brooklyn, N.Y. While investigating this story, she learned that she probably carries a genetic mutation that predisposes her to eczema.

