

CLOSE RELATIONSHIP has evolved between humans and *Helicobacter pylori*, the bacterium that can cause inflammation in the stomach and duodenum (red) but may actually protect the esophagus (green). Researchers believe the organisms exchange complex signals with human cells.

An Endangered Species in the Stomach

Is the decline of *Helicobacter pylori*, a bacterium living in the human stomach since time immemorial, good or bad for public health?

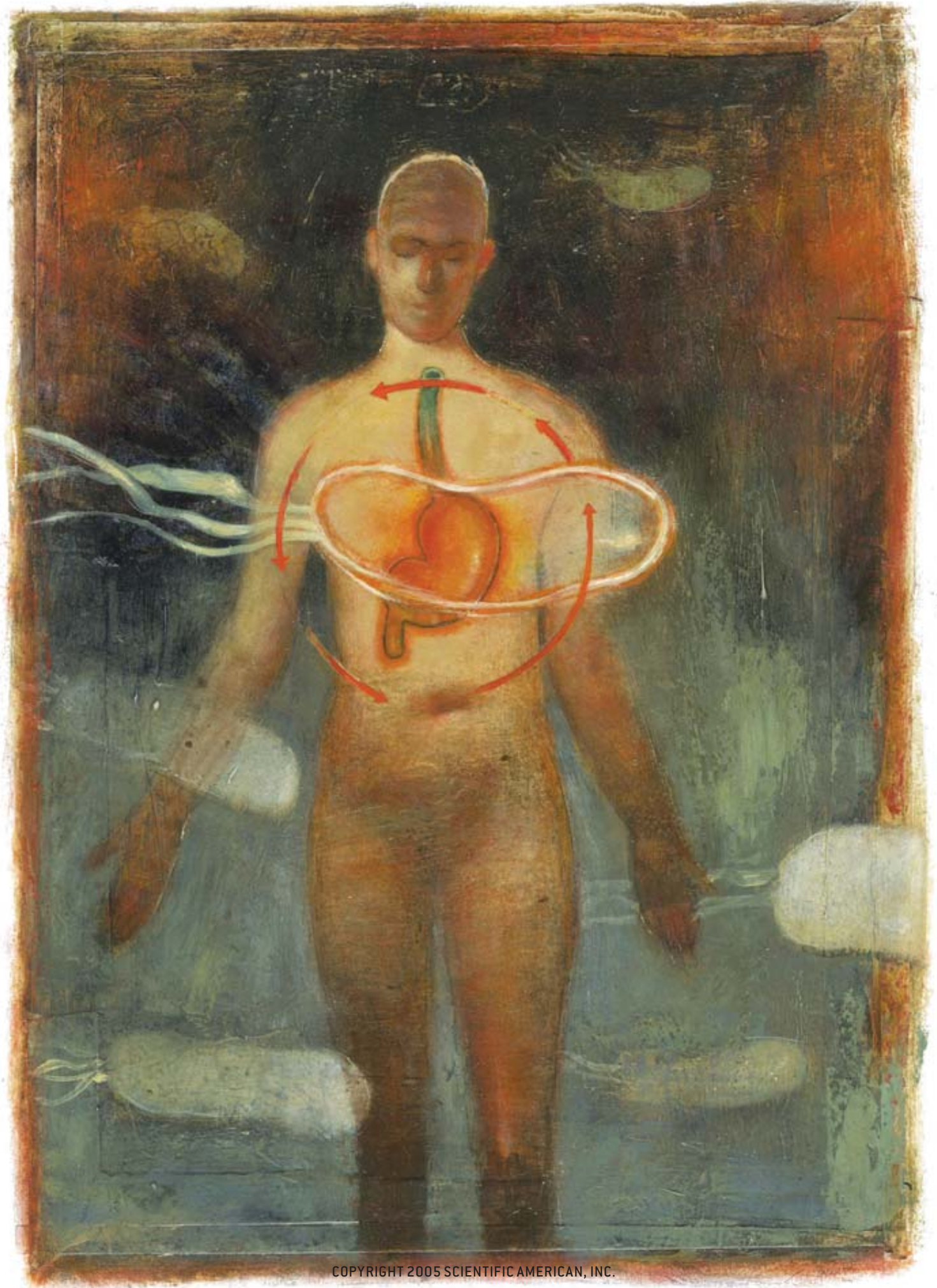
By Martin J. Blaser

Helicobacter pylori is one of humanity's oldest and closest companions, and yet it took scientists more than a century to recognize it. As early as 1875, German anatomists found spiral bacteria colonizing the mucus layer of the human stomach, but because the organisms could not be grown in a pure culture, the results were ignored and then forgotten. It was not until 1982 that Australian doctors Barry J. Marshall and J. Robin Warren isolated the bacteria, allowing investigations of *H. pylori*'s role in the stomach to begin in earnest. Over the next decade researchers discovered that people carrying the organisms had an increased risk of developing peptic ulcers—breaks in the lining of the stomach or duodenum—and that *H. pylori* could also trigger the onset of the most common form of stomach cancer [see “The Bacteria behind Ulcers,” by Martin J. Blaser; SCIENTIFIC AMERICAN, February 1996].

Just as scientists were learning the importance of *H. pylori*, however, they discovered that the bacteria are losing their foothold in the human digestive tract. Whereas nearly all adults in the developing world still carry the organism, its prevalence is much lower in developed countries such as the U.S. Epidemiologists believe that *H. pylori* has been disap-

pearing from developed nations for the past 100 years thanks to improved hygiene, which blocks the transmission of the bacteria, and to the widespread use of antibiotics. As *H. pylori* has retreated, the rates of peptic ulcers and stomach cancer have dropped. But at the same time, diseases of the esophagus—including acid reflux disease and a particularly deadly type of esophageal cancer—have increased dramatically, and a wide body of evidence indicates that the rise of these illnesses is also related to the disappearance of *H. pylori*.

The possibility that this bacterium may actually protect people against diseases of the esophagus has significant implications. For instance, current antibiotic treatments that eradicate *H. pylori* from the stomach may have to be reconsidered to ensure that the benefits are not outweighed by any potential harm. To fully understand *H. pylori*'s effects on health, researchers must investigate the complex web of interactions between this remarkable microbe and its hosts. Ultimately, the study of *H. pylori* may help us understand other bacteria that colonize the human body, as well as the evolutionary processes that allow humans and bacteria to develop such intimate relations with one another.



A Diverse Bacterium

AS SOON AS scientists began investigating *H. pylori*, it became clear that strains isolated from different individuals are highly diverse. (A variety of strains can also be found in a single stomach.) Although the strains are identical in appearance, their genetic codes vary greatly. Researchers have determined the complete genomic DNA sequences for two separate *H. pylori* strains; each has a single small chromosome of approximately 1.7 million nucleotides, comprising about 1,550 individual genes. (In comparison, *Escherichia coli*—a bacterium inhabiting the intestines—has about five million nucleotides, and humans have about three billion.) Remarkably, about 6 percent of the *H. pylori* genes are not shared between the two strains, and even the shared genes have a significant amount of variation in their nucleotide sequences.

This level of diversity within a species is extraordinary. The genetic differences between humans and chimpanzees—two distinct species—are tiny compared with the differences

The prevalence of *Helicobacter pylori* is much lower in developed countries such as the U.S.

among *H. pylori* strains: 99 percent of the nucleotide sequences in the human and chimp genomes are identical. The substantial variation in *H. pylori*'s genome suggests that either the bacteria have existed for a very long time as a species or that any particular variant is not so much better adapted to the human stomach as to outcompete all the others. In fact, both statements are true.

My laboratory has identified two particular types of variation. In 1989 we created a library of *H. pylori* genes by inserting selected fragments of the bacterium's DNA into cells of *E. coli*. The *E. coli* cells can then produce the proteins encoded by the *H. pylori* genes. We screened the resulting *E. coli* samples using blood serum from a person (me!) who carried *H. pylori* in his stomach; because my immune system had been exposed to the bacterium, the antibodies in my serum would

be able to recognize some of the organism's protein products. The first sample that my antibodies recognized contained a gene that we now call *cagA*, which encodes the CagA protein. This was the first *H. pylori* gene found in some but not all strains of the bacterium. Later research indicated that people infected with *H. pylori* strains bearing the *cagA* gene have a higher risk of acquiring peptic ulcer disease or stomach cancer than people with strains lacking the gene.

We now know that *cagA* is part of a region in the *H. pylori* chromosome that also contains genes encoding proteins that form a type IV secretion system (TFSS). Bacterial cells assemble these systems to export large, complex molecules into host cells; for example, *Bordetella pertussis*, the bacterium that causes whooping cough, uses a TFSS to introduce its toxin into the cells of the human respiratory tract. In 2000 research groups in Germany, Japan, Italy and the U.S. determined that several of the *H. pylori* genes near *cagA* encode TFSS proteins that assemble into a structure analogous to a

miniature hypodermic needle [see box on opposite page]. This structure injects the CagA protein into the epithelial cells that line the human stomach, which explains why my body produced antibodies to the protein.

After CagA enters an epithelial cell, enzymes in the host chemically transform the protein, allowing it to interact with several human proteins. These interactions ultimately affect the cell's shape, secretions and signals to other cells. Strains of *H. pylori* bearing the *cagA* gene cause more severe inflammation and tissue injury in the stomach lining than do strains without the gene. These differences may explain the increased disease risk in people carrying the *cagA* strains.

In the late 1980s Timothy Cover, then a postdoctoral fellow working with me, began to study some *H. pylori* strains that caused large holes, called vacuoles, to form in epithelial cells in culture. We showed that the active agent was a toxin, dubbed VacA, encoded by a gene that we named *vacA*. In addition to forming the vacuoles, VacA turns off the infection-fighting white blood cells in the stomach, diminishing the immune response to *H. pylori*. Unlike *cagA*, *vacA* is present in every *H. pylori* strain, but because the gene's sequence varies substantially, only some of the strains produce a fully functional toxin. John C. Atherton, a visiting postdoctoral fellow from England, found four major variations in *vacA*: two (m1 and m2) in the middle region of the gene and two (s1 and s2) in the region that encodes the protein's signal sequence, which enables the protein to move through cell membranes. Subsequent studies showed that the s1 variation could be divided into at least three subtypes: s1a, s1b and s1c.

H. pylori strains with both the m1 and s1 variations produce the most damaging form of the VacA toxin. Thus, it is

Overview/A Microbe's Effects

- Although *Helicobacter pylori* has long colonized human stomachs, improved sanitation and antibiotics have drastically cut the bacterium's prevalence in developed countries over the past century.
- People carrying *H. pylori* have a higher risk of developing peptic ulcers and stomach cancer but a lower risk of acquiring diseases of the esophagus, including a very deadly type of esophageal cancer.
- Studies of the interactions between *H. pylori* and humans may lead to better treatments for disorders of the digestive tract as well as a greater understanding of other bacteria that colonize the human body.

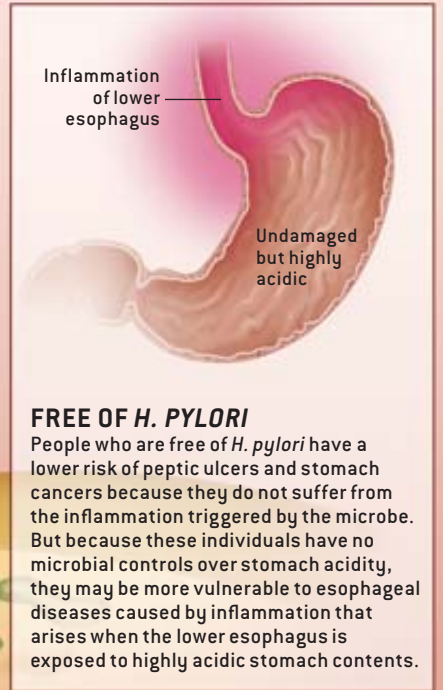
INTERACTIONS IN THE STOMACH

Helicobacter pylori can persist in the human stomach for decades, causing continual damage despite the host's immune response. Researchers theorize that the microbes

and host exchange signals in a negative feedback loop that moderates the tissue damage and maintains a stable environment for the bacteria.

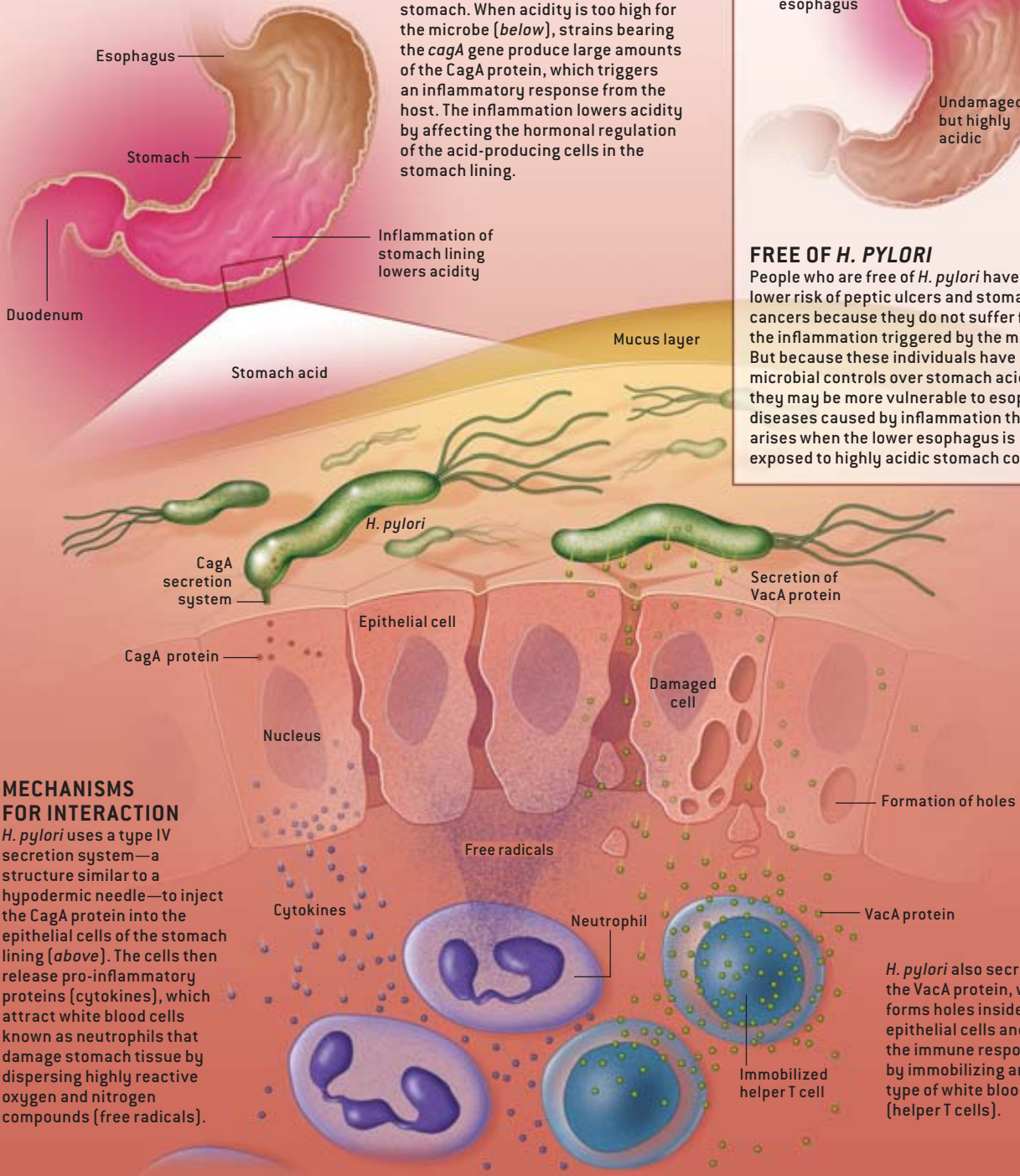
COLONIZED BY *H. PYLORI*

One example of *H. pylori*'s interactions is the use of inflammation (red tissue at left) to regulate acidity in the stomach. When acidity is too high for the microbe (below), strains bearing the *cagA* gene produce large amounts of the CagA protein, which triggers an inflammatory response from the host. The inflammation lowers acidity by affecting the hormonal regulation of the acid-producing cells in the stomach lining.



FREE OF *H. PYLORI*

People who are free of *H. pylori* have a lower risk of peptic ulcers and stomach cancers because they do not suffer from the inflammation triggered by the microbe. But because these individuals have no microbial controls over stomach acidity, they may be more vulnerable to esophageal diseases caused by inflammation that arises when the lower esophagus is exposed to highly acidic stomach contents.



MECHANISMS FOR INTERACTION

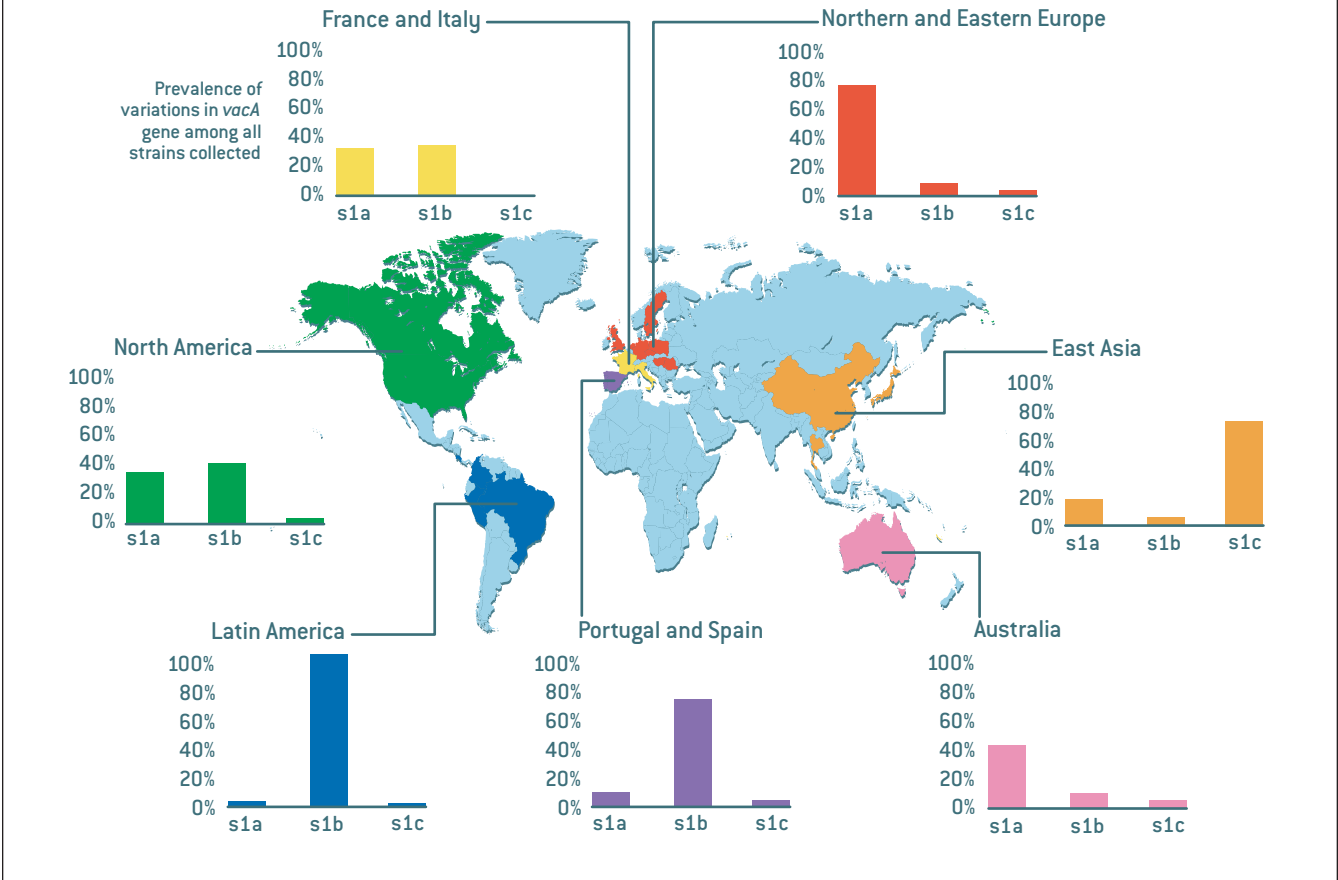
H. pylori uses a type IV secretion system—a structure similar to a hypodermic needle—to inject the CagA protein into the epithelial cells of the stomach lining (above). The cells then release pro-inflammatory proteins (cytokines), which attract white blood cells known as neutrophils that damage stomach tissue by dispersing highly reactive oxygen and nitrogen compounds (free radicals).

H. pylori also secretes the VacA protein, which forms holes inside the epithelial cells and curbs the immune response by immobilizing another type of white blood cell (helper T cells).

A WORLD OF VARIATIONS

H. pylori is an ancient and genetically diverse organism, and the geographic distribution of its variant strains reflects the origins and migrations of its human hosts. The s1a variation

of the *vacA* gene predominates in northern Europe, whereas the s1b and s1c variations prevail in the Mediterranean area and East Asia, respectively.



not surprising that strains bearing this genotype of *vacA*, combined with the *cagA* gene, are associated with the highest risk of stomach cancer. To make matters even more complicated, some people are more susceptible to these kinds of cancers because of variations in their own genes that enhance the inflammatory response to bacterial agents. The worst-case scenario is a person who carries the pro-inflammatory variations and is colonized with *H. pylori* strains containing the *cagA* gene and the s1/m1 *vacA* genotype. The collision of particularly aggressive *H. pylori* strains with particularly susceptible hosts appears to account for most cases of stomach cancer.

Tracing Migrations

ONCE SCIENTISTS HAD DISCOVERED ways to distinguish among the *H. pylori* strains that had been collected from around the world, they began to investigate whether strains circulating in different areas varied from one another. Working with Leen-Jan van Doorn of Delft Diagnostic Laboratory in the Netherlands, we found that variations in the *vacA* gene tended to cluster in certain geographic regions: s1c strains predominated in East Asia, s1a in northern Europe and s1b in the Mediterranean area [see box above].

My colleague Guillermo I. Perez-Perez and I were particularly interested in studying the *H. pylori* strains in Latin America because the results there could indicate when and how the bacteria arrived in the New World. We initially found that the Mediterranean strain, s1b, was by far the most common, suggesting that *H. pylori* was brought by Spanish and Portuguese settlers or African slaves. We realized, however, that these studies were conducted in Latin America's coastal cities, where the people have mixed European, African and Amerindian ancestries. Working with Maria Gloria Dominguez Bello of the Venezuelan Institute for Scientific Research, we analyzed stomach samples from a more indigenous Amazonian population—peo-

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ple in Puerto Ayacucho, a market town on the Orinoco River in Venezuela—and found that most of the strains had the *s1c* genotype that is prevalent in East Asia. This work provided evidence that *H. pylori* had been transported across the Bering Strait by the ancestors of present-day Amerindians and thus has been present in humans for at least 11,000 years.

More recent collaborations with Mark Achtman, Daniel Falush and their colleagues at the Max Planck Institute for Infection Biology in Berlin have shown that all modern *H. pylori* strains can be traced to five ancient populations—two arising in Africa, two in western or central Eurasia and one in East Asia. In fact, the genetic variations of *H. pylori* can be used to trace human settlement and migration patterns over the past 60,000 years. Because *H. pylori* is so much more ge-

ed, the incidences of both peptic ulcer disease (except those cases caused by aspirin and nonsteroidal anti-inflammatory agents such as ibuprofen) and stomach cancer are clearly declining in developed countries. Because these illnesses, especially stomach cancer, develop over many years, the drop in disease incidence has lagged several decades behind the decline in *H. pylori* infection, but the falloff is startling nonetheless. In 1900 stomach cancer was the leading cause of cancer death in the U.S.; by 2000 the incidence and mortality rates had fallen by more than 80 percent, putting them well below the rates for colon, prostate, breast and lung cancers. Substantial evidence indicates that the continuing extinction of *H. pylori* has played an important role in this phenomenal change. This is the good news.

The genetic variations of *H. pylori* can be used to trace human settlement and migration patterns.

netically diverse than *Homo sapiens*, the bacteria can better elucidate the history of population movements than can studies of human mitochondrial DNA (the most commonly used marker for such investigations). As researchers attempt to clock the migrations of our species, the mitochondrial studies may provide the hour hand, but the genetic sequences of *H. pylori* may offer a more accurate minute hand.

A Microbial Extinction

HUMANS ARE THE ONLY HOSTS for *H. pylori*, and the spread of the bacterium involves mouth-to-mouth or feces-to-mouth transmission. The geographic differences in *H. pylori* infection rates—much lower in the developed world than elsewhere—may be partly the result of improvements in sanitation in the U.S., Europe and other developed countries over the past century. But I believe that the widespread use of antibiotics has also contributed to the gradual elimination of *H. pylori*. Even short courses of antibiotics, given for any purpose, will eradicate the bacteria in some recipients. In developing countries where antibiotics are less commonly used, 70 to 100 percent of children become infected with *H. pylori* by the age of 10, and most remain colonized for life; in contrast, fewer than 10 percent of U.S.-born children now carry the organism. This difference represents a major change in human microecology.

Furthermore, the disappearance of *H. pylori* may be a sentinel event indicating the possibility of other microbial extinctions as well. *H. pylori* is the only bacterium that can persist in the acidic environment of the human stomach, and its presence can be easily determined by tests of blood, stool, breath or stomach tissue. But other body sites, such as the mouth, colon, skin and vagina, have complex populations of indigenous organisms. If another common bacterium were disappearing from these tissues, we would not have the diagnostic tools to detect its decline.

What are the consequences of *H. pylori*'s retreat? As not-

At the same time, however, there has been an unexpected rise in the incidence of a new class of diseases involving the esophagus. Since the early 1970s, epidemiologists in the U.S., the U.K., Sweden and Australia have noted an alarming jump in esophageal adenocarcinoma, an aggressive cancer that develops in the inner lining of the esophagus just above the stomach. The incidence of this illness in the U.S. has been climbing by 7 to 9 percent each year, making it the fastest-increasing major cancer in the country. Once diagnosed, the five-year survival rate for esophageal adenocarcinoma is less than 10 percent.

Where are these terrible cancers coming from? We know that the primary risk factor is gastroesophageal reflux disease (GERD), a chronic inflammatory disorder involving the regurgitation of acidic stomach contents into the esophagus. More commonly known as acid reflux disease, GERD was not even described in the medical literature until the 1930s. Since then, however, its incidence has risen dramatically, and now the disorder is quite common in the U.S. and other western countries. GERD can lead to Barrett's esophagus, a premalignant lesion first described in 1950 by English surgeon Norman Barrett. The incidence of Barrett's esophagus is rising in tandem



NATURAL HABITAT of *H. pylori* is the layer of mucus that lines the human stomach.

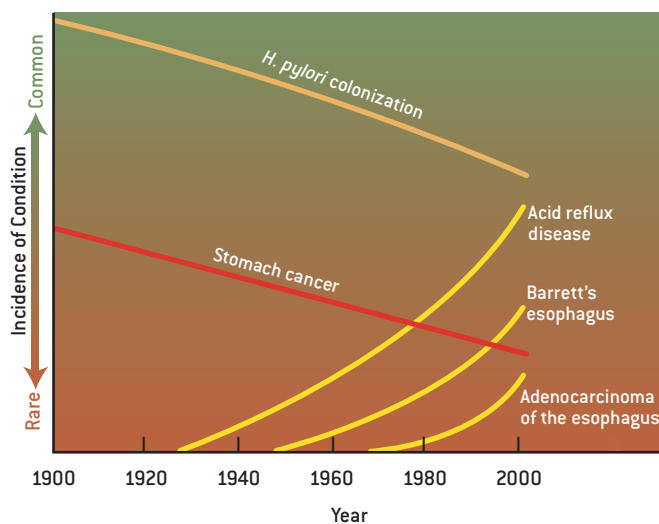
with that of GERD, and patients suffering from the condition have an increased risk of developing esophageal adenocarcinoma. It is becoming clear that GERD may initiate a 20- to 50-year process: in some cases, the disorder slowly progresses to Barrett's esophagus and then to adenocarcinoma, paralleling the gradual changes that lead to cancers in other epithelial tissues [see illustration at right]. But why are GERD and its follow-on disorders becoming more common?

The rise of these diseases has occurred just as *H. pylori* has been disappearing, and it is tempting to associate the two phenomena. When I began proposing this connection in 1996, I was greeted first by indifference and then by hostility. In recent years, though, a growing number of studies support the hypothesis that *H. pylori* colonization of the stomach actually protects the esophagus against GERD and its consequences. What is more, the strains bearing the *cagA* gene—that is, the bacteria that are most virulent in causing ulcers and stomach cancer—appear to be the most protective of the esophagus! In 1998, working with researchers from the National Cancer Institute, we found that people carrying *cagA* strains of *H. pylori* had a significantly decreased risk of developing adenocarcinomas of the lower esophagus and the part of the stomach closest to the esophagus. Then, in collaboration with investigators from the Cleveland Clinic and the Erasmus Medical Center in the Netherlands, we showed a similar correlation for both GERD and Barrett's esophagus. Independent confirmations have come from the U.K., Brazil and Sweden. Not all investigators have found this effect, perhaps because of differences in the methods of the studies. Nevertheless, the scientific evidence is now persuasive.

A Theory of Interactions

HOW CAN COLONIZATION by *H. pylori* increase the risk of stomach diseases but protect against esophageal disorders? A possible explanation lies in the interactions between the bacterium and its human host. *H. pylori* has evolved into a most unusual parasite: it can persist in a stomach for decades despite causing continual damage and despite the host's immune response against it. This persistence requires that virtually all the "up-regulatory" events that cause inflammation in the stomach tissue must be balanced by "down-regulatory" events that prevent the damage from worsening too rapidly. There must be an equilibrium between microbe and host; otherwise, the host would die rather quickly, and the bacteria would lose their home before getting a chance to propagate to another person. But how can two competing forms of life achieve this equilibrium? My hypothesis is that the microbe and host must be sending signals to each other in a negative feedback loop.

Negative feedback loops are common in biology for the regulation of cellular interactions. Consider, for example, the feedback loop involving glucose and the regulatory hormone insulin. After you eat a meal, glucose levels in the bloodstream rise and the pancreas secretes insulin. The insulin causes glucose levels to fall, which signals the pancreas to reduce insulin secretion. By modulating the peaks and valleys in glucose lev-



DECLINE of *H. pylori* in developed countries over the past 100 years has reduced the incidence of stomach cancer but may be triggering an upsurge in diseases of the esophagus. In some cases, acid reflux disease progresses to Barrett's esophagus (a premalignant lesion) and then to adenocarcinoma, a particularly deadly type of cancer. Because historical data on some disorders are incomplete, the plot lines show only general trends in disease incidence.

els, the system maintains a steady state called homeostasis. First described in the 19th century by French physiologist Claude Bernard, this concept has become the basis for understanding hormone regulation.

In essence, I took this idea one step further: the feedback relationship can involve microbial cells as well as host cells. Over the years, working with mathematicians Denise Kirschner of the University of Michigan at Ann Arbor and Glenn Webb of Vanderbilt University, our concepts of feedback have become more complex and encompassing. In our current formulation, the *H. pylori* population in a person's stomach is a group of extremely varied strains cooperating and competing with one another. They compete for nutrients, niches in the stomach and protection from stresses. Over the millennia, the long coevolution of *H. pylori* and *H. sapiens* has put intense selective pressure on both species. To minimize the damage from infection, humans have developed ways to signal to the bacteria, through immune responses and changes in the pressure and acidity in the stomach. And *H. pylori*, in turn, can signal the host cells to alleviate the stresses on the bacteria.

A good example of an important stress on *H. pylori* is the level of acidity in the stomach. Too much acid will kill the bacteria, but an extremely low level is not good either, because it would allow less acid-tolerant organisms such as *E. coli* to invade *H. pylori*'s niche. Therefore, *H. pylori* has evolved the ability to regulate the acidity of its environment. For example, strains bearing the *cagA* gene can use the CagA protein as a signaling molecule. When acidity is high, the *cagA* gene produces a relatively large amount of the protein, which triggers an inflammatory response from the host that lowers acidity by affecting the hormonal regulation of the acid-producing cells in the stomach lining. Low acidity, in contrast, curtails

the production of CagA and hence reduces the inflammation.

This negative feedback model helps us understand the health effects of *H. pylori*, which depend in large part on the intensity of the interactions between the bacteria and their hosts. The *cagA* strains substantially increase the risk of stomach cancer because they inject the CagA protein into the stomach's epithelial cells for decades, affecting the longevity of the host cells and their propensity to induce inflammation that promotes cancer. Strains lacking the *cagA* gene are much less interactive, so they do not damage the stomach tissues as severely. On the other hand, *cagA* strains effectively modulate acid production in the stomach, preventing acidity levels from rising too high. People who carry strains lacking the *cagA* gene have a weaker modulation of acidity levels, and people who are not colonized by *H. pylori* have no microbial controls at all. The resulting swings in stomach acidity may be central to the rise in esophageal diseases, which are apparently triggered by the exposure of the tissue to highly acidic stomach contents.

The absence of *H. pylori* may have other physiological effects as well. The stomach produces two hormones that affect

tion of the human microbiota, the organisms that share our bodies. Our microbiota are highly evolved for living within us and with each other. How likely is it that a newcomer, an unrelated strain of bacteria from outside the body, can successfully rechannel the pathways of interaction in a beneficial way? The existing organisms have survived strong and continuous selection, and this "home court advantage" usually enables them to reject and eliminate any strangers.

But a new day for probiotics may be coming. The key step will be gathering more knowledge of our indigenous microbiota and how they interact with us. I believe that complex interactions take place wherever microbes colonize our bodies (for example, in the colon, mouth, skin and vagina), but because of the array of competing organisms in those tissues, the relations are difficult to elucidate. *H. pylori*, though, largely excludes other microbes from the stomach. By the paradox of its great adaptation to humans and by the accident of its progressive disappearance during the 20th century, *H. pylori* may become a model organism for investigating human microecology.

The study of *H. pylori* may help us understand other bacteria that colonize the human body.

eating behavior: leptin, which signals the brain to stop eating, and ghrelin, which stimulates appetite. Eradication of *H. pylori* with antibiotics tends to lower leptin and increase ghrelin; in one study, patients who had undergone treatment to eliminate *H. pylori* gained more weight than the control subjects did. Could changes in human microecology be contributing to the current epidemic of obesity and diabetes mellitus (an obesity-related condition) in developed countries? If this research were confirmed, the implications would be sobering. Doctors might need to reevaluate antibiotic treatments that rid the stomach of *H. pylori* (and remove critical bacteria from other parts of the body as well). Although some of the consequences of eradication may be for the better (for example, a reduced risk of stomach cancer) other effects may be for the worse. The balance between good and bad may well depend on the patient's age, medical history and genetic type.

Probiotics

IF RESEARCHERS CONCLUDE that *H. pylori* would actually benefit some individuals, should physicians reintroduce the bacterium to these patients' stomachs? For more than 100 years, both medical scientists and laypersons have been searching for probiotics, microbes that can be ingested to aid human health. The earliest studies focused on the *Lactobacillus* species, the bacteria that make yogurt and many cheeses, but the effects of reintroduction were, at best, of marginal value. Researchers have largely failed to find any effective probiotics despite a century of trying.

One reason for this failure is the complexity and coevolu-

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MORE TO EXPLORE

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