The New Path to Preventing Ulcers

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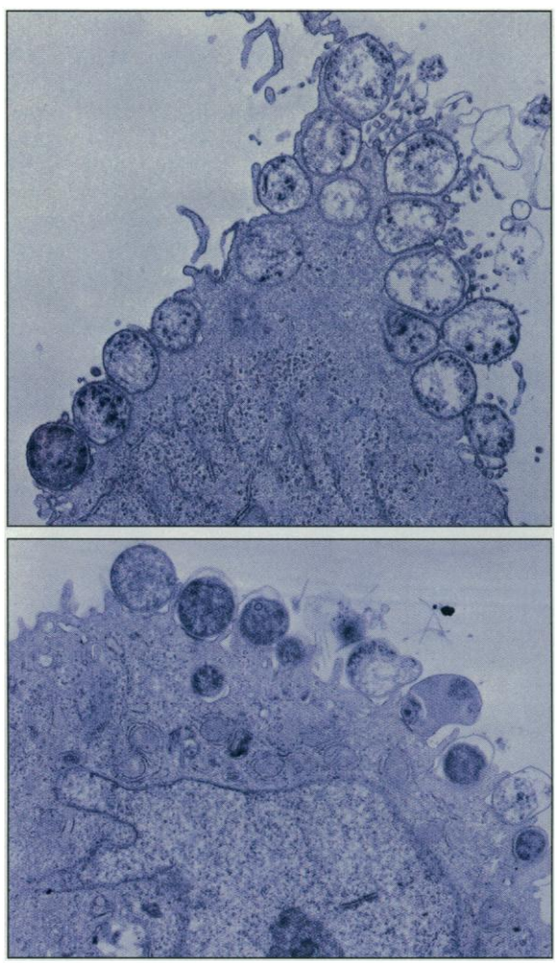
At its discovery in 1983, the association of a corkscrew-shaped bacteria with stomach ulcers (*1*), and subsequent identification of the bacteria as the new genus *Helicobacter*, was appreciated (and believed) by only a handful of microbiologists. Twelve years later, the close relation between *Helicobacter* infection, ulcers, and now stomach cancers has been ratified by a select panel of the National Insitutes of Health (*2*); gastroenterologists and the pharmaceutical industry are changing their treatment strategy for ulcers from reducing stomach acid to eradicating infection. Furthermore, *Helicobacter pylori* is now on the list of microbial agents that are classified as carcinogens because of its close association with two types of gastric cancers. Progress in understanding the pathogenesis of *H. pylori* infection has been hampered by the lack of suitable animal models to study the relatively rare occurrences of ulceration and cancer that accompany *Helicobacter* infection. Thus, the report by Marchetti *et al.* in this issue of *Science* (*3*), which describes a mouse model of persistent *Helicobacter* infection, provides a new opportunity to closely examine the interaction between *H. pylori* and a conveniently available mammalian host.

Infection of the stomach with *H. pylori* can occur as early as infancy, presumably through fecal-oral or salivary transmission, and can persist for the lifetime of its human host. Approximately 50 percent of the world's population is infected. Because crowding and low socioeconomic conditions favor transmission of this microbe, most children in developing countries become infected by the age of 10; in this setting, gastric cancer rates are very high and continue to increase. In the United States and other developed countries, standards of hygiene and the increasing socioeconomic status of the population have reduced the incidence of infection, and, in parallel, the rates of peptic ulcers and gastric cancer have declined. Nevertheless, ulcers and gastric cancer are serious health problems even in the United States, where vast sums of money are spent on ulcer remedies.



Close embrace. *Helicobacter pylori* attached to gastric epithlial cells, surrounded by cytoplasmic "arms." Some *H. pylori* have been internalized. [Electron micrographs by E. Segal and N. Ghori]

An intense medical campaign is being waged to cure infection in patients with documented ulcer disease and in patients at high risk for cancer. However, conventional antibacterial therapy, with combinations of antibiotics administered orally, is not necessarily cost-effective and has uncovered a propensity for *Helicobacter* to become resistant to antibiotics during therapy. Antibiotics are not a long-term answer; rather, we need to prevent infection, with a vaccine or other strategy.

We have begun to appreciate several of the invasive tactics employed by *H. pylori* to establish itself in the forbidding environment of the stomach. *Helicobacter* moves by five- or six-polar flagella into the mucous layer overlying the gastric epithelium. A potent urease produced by the microorganism is essential for colonization, presumably because it neutralizes the acidic environment (*4*). Although at any given time many free-swimming bacteria are seen in the gastric mucus, they also attach to gastric epithelial cells. A plethora of putative attachment factors have been described in *H. pylori* (*5*). A candidate receptor, Le^b, which is expressed on gastric epithelial cells, has been identified (*6*), and perhaps explains why individuals with blood group O are at higher risk for ulcer disease. The attachment of *H. pylori* to gastric epithelial cells resembles the attachment and effacing lesions of the enteropathogenic *Escherichia coli* (*7*), in which the organism sits on a pedestal of condensed actin and myosin. This suggests that the organism may be in intimate contact with the signal transduction machinery of the host cell, a proposal that is supported by the fact that host-specific phosphorylation of membrane proteins accompanies *H. pylori* attachment to cultured cells (*8*).

All *Helicobacter* species cause some degree of persistent inflammation when resident in the mammalian stomach. Gastritis is found in virtually all infected humans, although the majority have no symptoms. Only 1 in 10 will develop ulcer disease. In others, persistent inflammation eventually can lead to destruction of the normal epithelium, loss of the mucous layer, and increased cell turnover, a condition called atrophic gastritis and a serious risk factor for stomach cancer. Indeed, gastric adenocarcinoma is 3 to 12 times more likely to develop in individuals infected with *H. pylori* (*9*, *10*), and *H. pylori* infection has been linked to the development of low-grade, B cell lymphomas of gastric mucosa-associated lymphoid tissue (MALT) (*11*, *12*). What is the basis for these disparate clinical outcomes?

Many strains of *H. pylori* elaborate a potent cytotoxin, VacA, which produces vacuoles in cultured gastric cells that resemble the histological lesions in patient biopsy material. VacA is processed similarly to the immunoglobulin A protease of other mucosal pathogens like *Neisseria gonorrhoe-*

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ae, and its structure resembles that of a classic A-B bacterial toxin (*13*). The vacuoles produced by the purified toxin stain with cellular markers like Rab7, which are associated with the late endosome compartment of the endocytic pathway (*13*). Bafilomycin A1, which inhibits vacuolar-type H^+ adenosine triphosphatases, counteracts these effects and restores normal vesicle trafficking (*14*). This vacuolating action of VacA has been proposed to destroy the integrity of the gastric epithelium (*13*), although in the absence of an animal model of infection, the role of this protein in disease could not be fully confirmed. A second protein, CagA (cytotoxin-associated), is also associated with disease (*15*), but it is now clear that it has no cytotoxic activity nor is it necessary for cytotoxin expression (*16*).

Strains of *H. pylori* can be classified into two broad groups—those that express both VacA and CagA proteins (type I), and those that produce neither (type II) (*17*). Type I strains predominate in patients with ulcers (*13, 18*) and cancer (*19*). This finding is consistent with the observation that antibodies to CagA are associated with a higher degree of white cell infiltration of antral gastric mucosal cells and epithelial cell degeneration. Interleukin-8, a mediator of neutrophil migration, is up-regulated in patients infected with CagA-positive strains independent of VacA (*20*). The CagA gene and adjacent flanking DNA sequences are completely absent from the genome of most noncytotoxic strains of *H. pylori*. In spite of their lack of association with ulcers and cancer, type II strains still cause persistent inflammatory infection.

Further dissection of VacA and CagA functions requires an animal model. But because *H. pylori* has a narrow host range, much of our understanding of its pathogenesis is based on infection by other *Helicobacter* species in nonprimate models. These animal models have not been completely satisfactory because some *Helicobacter* species elicit a different type of inflammatory response than *H. pylori*. *Helicobacter* infections in germ-free or immunodeficient rodents do not produce a human type of inflammatory response. Furthermore, some of the *Helicobacter* species do not even contain the VacA- or CagA-associated genes.

The research group reporting the new mouse model (3) previously treated mice acutely with *H. pylori* to examine the role of VacA- and CagA-associated factors in the pathogenesis of the disease. They found that the cytotoxins produced a direct vacuolation of cells and ulceration of the stomach lining, whereas the CagA-associated factors were linked to the inflammatory process. The new model of persistant infection reported in this issue of *Science* permits the researchers to establish that type I strains of *H. pylori* from humans cause erosive lesions accompanied by inflammation, whereas type II strains colonize animals but elicit only a mild inflammatory response without ulceration.

Further, and of extreme practical importance, mice orally immunized with *Helicobacter* antigens, including VacA and urease, were protected from subsequent challenge with viable type I *H. pylori* strains, a finding previously inferred from an *H. felis* infection model (*21, 22*). These results suggest not only that vaccination might indeed prevent *Helicobacter* infection, but also that it provides a relatively cheap and simple model to identify other factors, including the CagA-associated gene products, that may be useful as vaccine antigens. Thus, urease, flagella proteins, and one or more *H. pylori* adhesins, as well as CagA and VacA, can be productively examined as potential vaccine candidates in this model.

Helicobacter has been called the "slow virus" of the bacterial world (*23*). How does this remarkable microorganism manage to infect most of us for a lifetime, causing a chronic inflammatory process that persists even in the face of a brisk humoral antibody response? The new animal model will allow us to delve more deeply into the pathogenesis of and cellular immune response to this unusual organism and, perhaps, to other persistent infectious agents.

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