Host-Bacterial Interactions in *Helicobacter pylori* Infection

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*Helicobacter pylori* are spiral-shaped gram-negative bacteria with polar flagella that live near the surface of the human gastric mucosa. They have evolved intricate mechanisms to avoid the bactericidal acid in the gastric lumen and to survive near, to attach to, and to communicate with the human gastric epithelium and host immune system. This interaction sometimes results in severe gastric pathology. *H pylori* infection is the strongest known risk factor for the development of gastroduodenal ulcers, with infection being present in 60%–80% of gastric and 95% of duodenal ulcers.1 *H pylori* is also the first bacterium to be classified as a definite carcinogen by the World Health Organization’s International Agency for Research on Cancer because of its epidemiologic relationship to gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma.2 In the last 25 years, since *H pylori* was first described and cultured, a complete paradigm shift has occurred in our clinical approach to these gastric diseases, and more than 20,000 scientific publications have appeared on the subject. From the medical point of view, *H pylori* is a formidable pathogen responsible for much morbidity and mortality worldwide. However, *H pylori* infection occurs in approximately half of the world population, with disease being an exception rather than the rule. Understanding how this organism interacts with its host is essential for formulating an intelligent strategy for dealing with its most important clinical consequences. This review offers an insight into *H pylori* host-bacterial interactions.

*Helicobacter pylori* infection is usually acquired early in childhood and lasts for a lifetime, and the majority of those infected (80%–90%) will carry and transmit *H pylori* without any symptoms of disease. In many ways, *H pylori* can be defined as a commensal and not a pathogen, making it difficult to determine who should be treated to prevent serious sequelae.3 Many clinical and basic research studies have been undertaken to define what makes *H pylori* a pathogen. It is now clear that both bacterial virulence factors and host susceptibility play a role. However, as investigators explore the intricate relationship between *H pylori* and the gastric mucosa at the cellular and molecular level, we find that the pathogenesis of *H pylori* is much more complex than simply defining how it “damages” the stomach.

In this review, we will discuss recent findings and concepts underlying the “virulence” mechanisms of *H pylori* pathogenesis but discuss them within the context of their known biologic properties, their role in the bacterial life cycle, and what these virulence factors have told us about the biology of the human stomach. We will also discuss how host risk factors for disease illustrate biologic principles of the function of the gastric mucosa that were previously unrecognized.

**Bacterial Factors**

*Transmission and Entry*

The very large number of people colonized throughout the world suggests that *H pylori* evolved a very robust strategy of transmission. However, our understanding of how *H pylori* infections are acquired and which bacterial factors are involved in transmission is limited. Unlike many organisms that have free-living forms in environmental reservoirs and unlike those that can infect many different animal and plant species, natural *H pylori* infection is restricted to humans and closely related primates. (Other species of gastric *Helicobacters* have been described in many other mammals ranging from cheetahs to polar bears and dolphins.4–6)

Epidemiologic studies of *H pylori* transmission show that the majority of infections tend to occur within...
families through close person-to-person contact. The close association between \( H \text{ pylori} \) and humans, and the fact that \( H \text{ pylori} \) strains are genetically divergent, has allowed investigators to ask questions about human evolution by studying the genetic makeup of \( H \text{ pylori} \) strains. For example, patterns of human migration have been mapped by genotyping the \( H \text{ pylori} \) strains found in different populations.7,8

Most \( H \text{ pylori} \) transmission occurs in childhood, and, in some countries, up to 90% of children become infected by age 10 years, with reports of infection as early as the first months of life.9 Maternal-to-child and sibling-sibling transmission seem most likely because longitudinal studies have shown that the risk of acquiring the infection is highly correlated to the infection status of the mother10 and siblings11 and related to overcrowding conditions in the home.12

Many sources of potentially infective \( H \text{ pylori} \) have been suggested, based on the finding that \( H \text{ pylori} \) DNA can be readily detected in a variety of places, including sewage and water sources. However, the isolation of viable \( H \text{ pylori} \) from any of these sources is extremely rare.13 Some investigators propose that \( H \text{ pylori} \) may exist in the environment in a dormant, spore-like state that can be viable but nonculturable. This hypothesis partially comes from the observation that, under stress and nutrient deprivation, \( H \text{ pylori} \) undergoes a morphologic transformation from actively dividing and swimming spiral bacilli to inactive cocci. However, until now, no definitive experimental evidence exists that \( H \text{ pylori} \) can revert from a coccoid form into the infectious spiral bacilli. In contrast, there is ample experimental evidence that culturable bacilli are infectious in animal models, in volunteer studies,14 and in reports of inadvertent transmission through contaminated endoscopes.15

It, therefore, seems more likely that transmission occurs when \( H \text{ pylori} \) is present outside of the stomach in a culturable form. However, we also know that \( H \text{ pylori} \) is fragile outside of the human stomach because it is rapidly killed by higher oxygen tension and even by light.16 The most likely modes of transmission seem to be situations in which gastric contents can be transferred quickly from person to person. Gastric-oral transmission is suggested in association with gastroenteritis with vomiting. Oral-oral transmission is also possible if \( H \text{ pylori} \) can survive for short periods after gastric contents have refluxed into the oral cavity. Fecal-oral transmission may be possible under conditions in which \( H \text{ pylori} \) survive transit through the lower gastrointestinal tract, which is uncommon in healthy people.17-20

An experimental study in humans confirmed that \( H \text{ pylori} \) is not typically culturable in normal human feces, but, when diarrhea is induced in naturally infected volunteers, up to 20% of stool samples contain culturable \( H \text{ pylori} \).19 The same study showed that when emesis is induced in infected volunteers, all samples of vomitus contain culturable \( H \text{ pylori} \), often in high numbers. A follow-up epidemiologic study shows that \( H \text{ pylori} \) transmission correlates with exposure to an infected family member with a bout of gastroenteritis. The highest risk of acquiring infection was found in association with vomiting.17 In accord with these studies, an experimental study in rhesus monkeys showed that young monkeys become rapidly infected if they are allowed contact with other infected adults and that \( H \text{ pylori} \) can be consistently cultured from vomitus, whereas culture from feces, saliva, or the environment is very rare.20

So far, specific \( H \text{ pylori} \) factors have not been studied in the context of transmission for lack of readily available experimental models. One aspect that has received attention and debate is whether the coccoid form of \( H \text{ pylori} \) is viable or simply a form of bacterial death. A recent report found that the \( H \text{ pylori} \) cell wall enzyme AmiA, a peptidoglycan hydrolase, is involved in the morphologic transition from spiral to coccoid, suggesting that the coccoid transition might be a regulated process rather than a degenerate form of \( H \text{ pylori} \).21 Interestingly, this study21 showed that the peptidoglycan of the coccoid form is a poor activator of the innate immune response, and the authors suggest that the transition into coccoid may represent remodeling of the cell wall for the purpose of immune modulation. Nagai et al recently observed that the coccoid form of \( H \text{ pylori} \) is phagocytosed by dendritic cells in Peyer’s patches.22 They proposed that \( H \text{ pylori} \) converts to the coccoid form in the anaerobic small intestine and stimulates the host immune system through Peyer’s patches. Although the speculation that the coccoid form of \( H \text{ pylori} \) may act to protect the rest of the population from immune attack remains to be tested, the notion that \( H \text{ pylori} \) exists in many forms that are dynamic and adapt to changing conditions in the gastric mucosa is a recurrent theme in \( H \text{ pylori} \) biology.

**Colonization of the Mucous Layer**

\( H \text{ pylori} \) is the only known organism capable of colonizing the harsh environment of the human stomach, but it dies rapidly in the low pH found in the stomach lumen. To avoid the bactericidal activity of acid, \( H \text{ pylori} \) generate large quantities of cytosolic and cell surface-associated urease, an enzyme capable of transiently buffering the acidic environment by the breakdown of urea to generate ammonia and carbon dioxide. This enzymatic activity is highly conserved in different \( H \text{ pylori} \) strains and therefore useful for diagnostic purposes. The urea breath test detects labeled carbon dioxide that is generated by urease metabolism of urea. \( H \text{ pylori} \) also possess urease-independent mechanisms to adapt to mild acidic conditions and use several stress responses to transiently withstand acid.23,24 (Figure 1). However, even with multiple mechanisms of acid adaptation, \( H \text{ pylori} \) remains susceptible to acid and can only survive for a few minutes at low pH. Instead of becoming an acidophile, \( H \)}
Helicobacter pylori has evolved several strategies to minimize exposure to the low pH in the stomach lumen by remaining in very close proximity to the surface of the epithelium where the pH is near neutral.

Most H pylori bacilli are free swimming within a narrow band of the protective mucus gel that is constantly being secreted and renewed.25 To remain in the mucus layer, H pylori need to utilize their polar flagella for motility. Both the ability to swim with flagellar motion and the ability to control the direction of movement by chemotactic responses are essential for H pylori colonization.26,27 A study of the spatial orientation of H pylori within the gastric mucus layer in Mongolian gerbils showed that they actively remain within 25 μm of the surface of the epithelium and orient themselves based on the pH gradient in the mucus, avoiding the acidic distal regions.28 If these pH gradients are eliminated experimentally, H pylori lose their spatial organization, and, if the bacteria are placed at pH 4 or lower, they lose their motility in a matter of minutes.29 It, therefore, seems that a constant sensing and responding to pH gradients is critical for the survival of the bacteria in the stomach (Figure 1). A recent report identified an H pylori chemoreceptor responsible for the negative chemotaxis in pH gradients. H pylori increase their swimming speed when placed near an acidic gradient.30 They also change their swimming paths to favor movement away from acid. An H pylori mutant in the tlpB gene, which codes for a chemoreceptor, can swim but do not move away from acidic regions. These mutant H pylori are defective in their ability to colonize mice stomachs.30

The active sensing and swimming away from the gastric lumen bring large numbers of H pylori into repeated close contact with the epithelial surface. It has been estimated that colonization densities may reach as high as 100 million bacteria/mL of stomach mucus.31

### Interactions With the Gastric Epithelium

#### Adherence

Approximately 20% of H pylori in the stomach are found adhered to the surfaces of mucus epithelial cells.32 Electron microscopy studies of gastric biopsy specimens have also shown that H pylori adhesion includes a specific tropism for the intercellular junctions, and bacteria are also sometimes seen deep in the intercellular spaces, associated with cytoskeletal changes at the adhesion site, or sometimes internalized into epithelial cells.33–37 All these observations suggest that adhesion involves specialized molecular interactions with the gastric mucosa that may lead to intimate attachment and modification of the cell surface and underlying cytoskeleton.

The genomic sequences of several H pylori strains are now available and show that, despite their relatively small genomes, H pylori have over 30 genes dedicated to the expression of outer membrane proteins. Several of these have been classified as adhesins, suggesting multiple and perhaps redundant or variable modes of attachment to the cell surface. The best studied H pylori adhesins are outer membrane proteins that bind carbohydrate modifications in host cell glycopolypetides. The adhesin BabA binds the fucosylated blood group antigen Lewis-b.38 The outer membrane protein SabA adheres to sialeted glycoproteins, specifically to sialyl-Lewis-X.39 HopZ and HopH (OipA) have also been proposed to act as adhesins based on mutagenesis studies that show they partially mediate binding to AGS gastric adenocarcinoma cells in culture, although their receptors have not yet been character-
ized.\textsuperscript{40–42} The AlpA and AlpB proteins have also been defined as adhesins in vitro, and mutants in these genes show defects in colonization of guinea pig stomach.\textsuperscript{43}

From the perspective of the host, a recent report showed that \textit{H pylori} can adhere to cells via decay-accelerating factor (DAF), a host cell glycosylphosphatidylinositol (GPI)-anchored glycoprotein that is normally involved in protecting cells from complement activation on their surfaces.\textsuperscript{44} A region of DAF is glycosylated, and \textit{H pylori} attachment to DAF requires this glycosylated domain; however, the \textit{H pylori} adhesin involved in binding DAF has not yet been determined. Interestingly, binding of \textit{H pylori} to cells in culture, up-regulated expression of DAF, and mice genetically deficient in DAF had less gastric inflammation despite being equally colonized with \textit{H pylori} than control littermates. The attachment to DAF is also intriguing in that the group B coxsackieviruses were recently shown to co-opt DAF binding to breach the epithelial barrier and reach its receptors for invasion at the tight junctions. Binding of DAF on the apical cell surface activates the c-abl kinase, triggering Rac-dependent actin rearrangements that permit virus movement to the tight junction.\textsuperscript{45} Because \textit{H pylori} has tropism for the junctions, and also activates src and abl kinases via CagA injection (see below), it will be interesting to determine whether there is a link between DAF adhesion and the junctions in \textit{H pylori} biology.

Several recurrent themes in the biology of \textit{H pylori} adhesins exemplify the importance of heterogeneity in this system.\textsuperscript{46} First, no individual adhesin is essential for attachment to the gastric mucosa, indicating redundancy of adhesive mechanisms. Second, expression of adhesins is diverse between strains and variable within a single strain over time, and these mechanisms of variability and adaptation are controlled at the genetic level by on/off switching of adhesin gene expression, gene inactivation, or recombination.\textsuperscript{41,47,48} An individual adhesin may show dynamic adaptation with variable affinities for its receptor, perhaps as a mechanism of adapting to changes in mucosal glycosylation at different anatomic locations and over time.\textsuperscript{49} Finally, adhesive interactions contribute to inflammation and are likely involved in disease progression.\textsuperscript{59,50,51}

**Why Does \textit{H pylori} Adhere to Cell Surfaces?**

Three main hypotheses that are not mutually exclusive have been presented in regards to this question.

**Adhesion to cause cellular damage and inflammation.** The first hypothesis is that \textit{H pylori} adheres to cause damage to the epithelium, induce inflammation, and deliver toxins. In particular, SaBA has been shown to bind to inflamed mucosa and also to activate neutrophils through selectin mimicry.\textsuperscript{51} There is also experimental evidence linking bacterial adhesion and disease. When transgenic mice expressing the Lewis-B antigen were infected with \textit{H pylori}, the mice showed increased bacterial attachment, more severe chronic gastritis, and parietal cell loss.\textsuperscript{52} The delivery of the major \textit{H pylori} virulence factors CagA and VacA are intimately related to adhesion as well, suggesting that a major role of adhesion is the delivery of toxins. Several investigators have speculated that increased inflammation and damage caused by adhesion are of adaptive value to \textit{H pylori} because of the release of nutrients into the gastric lumen. Although unproven, the hypothesis that inflammation is beneficial to \textit{H pylori} is intriguing, given that inflammatory changes accompany most if not all \textit{H pylori} infections regardless of symptoms.

**Adhesion to avoid mechanical clearance and promote invasion and persistence.** A second hypothesis to explain \textit{H pylori} adhesion is that it evolved as a way to avoid mechanical clearance. The gastric environment is clearly dynamic and has several mechanisms of clearance, including the constant exocytosis of mucopolysaccharides, the secretion of gastric juices, and peristaltic movement of the gastric walls. Under conditions of flow, bacterial pathogens and commensals that colonize mucosal surfaces have developed numerous adhesive mechanisms, such as pili or fimbriae to avoid being cleared from the mucosa.\textsuperscript{53} \textit{H pylori} may derive a transient foothold through adhesion to the epithelial cell surface, especially if the clearance mechanisms of the stomach sometimes overwhelm the free-swimming population.

A second way to avoid clearance is to invade mucosal epithelial cells and thus remain mechanically attached and also protected from the extracellular environment. For example, some microbes such as uropathogenic \textit{Escherichia coli}, which are largely extracellular organisms, are not only able to adhere to bladder epithelial cells but also invade cells to establish a persistence intracellular niche and avoid mechanical clearance.\textsuperscript{54} Similarly, a small proportion of \textit{H pylori} are also found invading the epithelial barrier, either between cells or within epithelial cells.\textsuperscript{37} In cell culture experiments, intracellular \textit{H pylori} have been shown to remain viable inside cells for several days, even in the presence of extracellular antibiotics, and to be able to repopulate the extracellular environment.\textsuperscript{55} Although, currently, there is no evidence that \textit{H pylori} can replicate intracellularly, and no specific \textit{H pylori} factors have been found to be essential for cell invasion, clinical evidence shows that \textit{H pylori} infections cannot be eradicated with antibiotics that have poor intracellular penetration, suggesting that even small numbers of bacteria breaching the epithelial barrier could have an important role in persistence of the organism.\textsuperscript{56}

**Use of the cell surface as a site of replication.** A third possibility is that \textit{H pylori} not only transiently attaches to the surface of the epithelium but actually uses the cell surface as a site of replication. No experimental studies have yet directly explored the possibility that \textit{H pylori} actively divide while attached to the cell surface, but a recent study showed that \textit{H pylori} is capable of obtain-
ing cholesterol directly by attachment to the host cell and that it incorporates and modifies this cholesterol within its own cell membrane.57

Two Major Virulence Factors That Co-opt Epithelial Cell Functions

Some of the events that lead to chronic inflammation and disease of the gastric mucosa are probably secondary to immune recognition of the colonizing bacteria and inadvertent damage from various bacterial products. For an excellent account of the immunology of H pylori infection please refer to the review by Wilson and Crabtree published in this series earlier in 2007.58 Some H pylori products have been identified in the context of disease or can be classified as “toxins” by biologic activity. We will discuss the 2 best characterized virulence factors of H pylori: the cytotoxin VacA and the cag pathogenicity island and its effector CagA (Figure 1).

VacA

VacA is a pore-forming cytotoxin that was identified when the supernatants of H pylori broth cultures were found to cause aberrant vacuolation of cultured cells.59 As with most H pylori factors, there is considerable genetic diversity in the vacA gene, and, therefore, the activities of different alleles of the toxin differ in their cytotoxicity. For VacA to intoxicate cells, it must be secreted from the bacteria and delivered in an active form to host cell membranes at which it assembles into pores that allow the leakage of chloride ions.60

VacA is first made as a large 140-kilodalton polypeptide that is trimmed at both ends during secretion from the bacterial cell. The amino terminus contains a signal sequence (or “s” region of the gene) that shows allelic variability and has been classified into different types. Strains harboring s1 types of VacA secrete active toxin and are also more highly associated with both ulcers and gastric cancer.61 The carboxyl end of the pro-VacA peptide is involved in the autotransport of the toxin out of the bacterial outer membrane and is also removed from the mature toxin.62 The middle region of the gene is classified as the “m” region, which also shows allelic variation, with m1 subtypes having stronger vacuolating activity. Most of the biochemical studies have been performed with s1m1 variants of VacA.

The mature VacA protein is 88 kilodaltons but can be further nicked into 2 smaller peptides that remain noncovalently associated.63 Upon secretion, approximately 50% of the toxin remains associated with the bacterial cell surface, and the rest is released. Interestingly, the VacA molecules that remain associated with the bacterial surface are functional and are delivered to host cells by direct contact between adhered bacteria and the host cell membrane.64 The free protein in the supernatant oligomerizes into molecular complexes that form a ring structure with the shape of a flower.65 It is not clear whether these free VacA complexes are toxigenic because they are biologically inactive unless they are dissociated by high or low pH into the monomeric forms that can be taken up by cell membranes. It is believed that the flower-like ring complex of VacA is the conformation it adopts when inserted into membranes, hence its pore-forming properties.67

VacA has several toxigenic properties that may alter the outcome of infection and colonization by H pylori. The best studied is VacA’s effect on endosomal maturation leading to vacuolation of epithelial cells. It is believed that VacA inserts into the membranes of late endosomal vesicles, forms pores with chloride channel activity, alters the composition of anions within endosomes, and subsequently leads to osmotic swelling. Although it is not clear whether this is the major role of VacA in vivo, alterations in endosomal function in the gastric mucosa could have many effects on the epithelium. For example, antigen presentation depends on proper function of endosomal trafficking, and VacA has been shown to perturb antigen presentation in vitro.68 In addition to its effects on endosomes, VacA also induces host-cell death through apoptosis. This is thought to occur through pore formation in mitochondrial membranes69 and also indirectly through the activation of proapoptotic signaling molecules.70 Purified VacA can cause epithelial erosions when applied directly to the mouse gastric mucosa. One putative cell surface receptor for VacA is the protein tyrosine phosphatase receptor type Z (Ptprz). Interestingly, Ptprz was found to mediate the erosive effects of purified VacA on the mucosa because mice genetically deficient in Ptprz were resistant to the ulcerogenic injury of VacA.71 A third reported effect of VacA is its ability to cause leakage of ions and small molecules, such as iron, nickel, sugars, and amino acids, by disrupting the barrier function of tight junctions, without major disruptions in junction integrity. This could be a mechanism by which H pylori acquires nutrients across an intact epithelial barrier.72 Recent studies have also focused on the potential effects of VacA on the immune system. VacA was found to be a very powerful inhibitor of T-cell activation in vitro.73

The role of VacA in vivo is not well understood. It has been speculated that VacA may allow H pylori to acquire nutrients by damaging the epithelial barrier or causing paracellular leakage of small molecules. It is also possible that its main role is in suppressing the T-cell immune response, although it has not been established whether enough VacA penetrates beyond the epithelium for this purpose. It is also not known whether the adhesion-mediated delivery of VacA is more important than the role of soluble VacA, given the tendency of the monomers to oligomerize into inactive structures in solution.

Given the universal presence of the vacA gene in H pylori strains, its genetic variability, and its various effects on host cells, it seems likely that it plays an important role in the life of H pylori within the human mucosa.
Although mutant *H pylori* strains lacking the *vacA* gene are no different from wild-type bacteria when grown in vitro, 2 studies have shown effects of *VacA* during infection in vivo. In a study of *H pylori* infection in Mongolian gerbils, *vacA*-deficient *H pylori* caused similar inflammation than those infected with wild-type isogenic bacteria, but the animals colonized by *vacA* mutant strains showed statistically significantly less gastric ulceration.\(^7^4\) In an independent experiment in which mice were infected with *H pylori* as a mixture of wild-type and *vacA* gene deletion mutants, the wild-type bacteria out competed the *VacA*-deficient strain, indicating a benefit for *VacA* in colonization at the individual bacteria level.\(^7^5\)

**CagA and the cag Pathogenicity Island Type 4 Secretion System**

CagA is an *H pylori* protein that was initially found as a marker for disease because, in some populations, patients with antibodies against this protein show higher rates of both peptic ulcers\(^7^6\) and gastric adenocarcinoma.\(^7^7\) The relative risk associated with CagA+ infection varies in different studies, with a few studies failing to find the association. Most show increased odds ratios in the 2–3 range, but some studies show increased risk of cancer with CagA+ *H pylori* infections with odds ratios as high as 28.4 (95% CI: 3.7–217.1).\(^8^0\) CagA+ strains have also been associated with increased inflammation,\(^8^1\) cell proliferation,\(^8^2\) and metaplasia\(^8^3\) of the gastric mucosa.

The reason CagA is associated with disease and its purpose in the life of *H pylori* are still not completely understood. However, the cellular biology of CagA and its ability to activate signaling mechanisms and affect the structure, differentiation, and behavior of epithelial cells has become a fascinating area of investigation, illustrating how microbial molecules evolve to control host cell function. We currently know that CagA can activate a number of signal transduction pathways that resemble signaling by growth factor receptors, and, simultaneously, CagA is involved in binding and perturbing the function of the epithelial junctions, resulting in aberrations in tight junction function, cell polarity, and cellular differentiation.

**The cag pathogenicity island and the H pylori needle.** The first major breakthrough in the study of CagA was the realization that its gene is part of a large pathogenicity island, a region of horizontally acquired DNA that was inserted into the genome of the more virulent *H pylori* strains. It is not known where the cag pathogenicity island (cag PAI) arose or how *H pylori* acquired it, and CagA has no homology to other known proteins. However, many of the genes adjacent to cagA have significant homology to components of a type 4 secretion system (TFSS), which code for macromolecular structures that function as minute needles for the transfer of bacterial products from pathogenic bacteria into host cells. The best studied TFSS is that of the plant pathogen *Agrobacterium tumefaciens*.\(^8^4\) These bacteria inject a DNA plasmid into host plant cells to induce the formation of a plant tumor or gall. It was thus hypothesized that the cag PAI of *H pylori* could serve as a novel transport system for secretion of virulence factors.\(^8^5\)

**CagA is injected into the host.** The next major breakthrough in understanding the function of CagA came when investigators noted that, upon contact with gastric cancer cells in culture, some *H pylori* strains are capable of causing significant cytoskeletal rearrangements and changes in cell shape, and these changes are associated with tyrosine phosphorylation of certain proteins.\(^8^6\),\(^8^7\) Because tyrosine phosphorylation is known to be an important mechanism of eukaryotic cell signaling, it was initially believed that *H pylori* attachment triggered the phosphorylation of host cell proteins. However, when several groups succeeded in identifying the major protein being phosphorylated, it was found that it was not of host origin but rather CagA.\(^8^8\)–\(^9^2\) This was a very surprising finding because it implied both that CagA is transferred into the host cell cytoplasm and that CagA can interact with tyrosine kinase enzymes within the host. Mutations in the genes coding for the TFSS abrogate CagA delivery without affecting its expression within the bacteria, indicating that CagA is delivered to the host through this injection device. The cag TFSS has been visualized and is beginning to be characterized at a molecular level, but much remains to be learned about how and when CagA is translocated into the host. To date, CagA is the only bacterial effector protein known to be translocated by the cag TFSS, although bacterial peptidoglycan may also leak into the cell through the T4SS and activate Nod1-mediated inflammatory responses.\(^9^3\)

**CagA is phosphorylated by cellular oncogenes.** The c-terminal tail of the CagA protein varies among *H pylori* strains and is the target region for phosphorylation.\(^9^4\) The phosphorylated tyrosines are contained within the EPIYA amino acid motif. These motifs are variable in number and organization in the cagA genes from different strains of *H pylori*, which have and may partially explain differences in the pathogenicity of different *H pylori* strains.\(^9^5\),\(^9^6\) Interestingly, the cellular enzymes responsible for phosphorylation of CagA are known oncogenes. Several studies have identified src-family kinases, which are normally involved in controlling basic cytoskeletal processes, cell proliferation, and differentiation, but also are key players in carcinogenesis, as the kinases that activate CagA.\(^9^4\) More recently, another tyrosine kinase involved in oncogenesis, c-abl, was found to also phosphorylate CagA.\(^9^7\)

**CagA stimulates growth factor receptor-like signaling.** After its tyrosine phosphorylation, CagA remains near the plasma membrane. There, it interacts with a number of host proteins, triggering signals that resemble the activation of receptor-tyrosine kinase growth factors
CagA in vivo. We have learned a great deal about the molecular pathways activated by CagA; however, we know relatively little of which of these events occur in vivo in the stomach, which are more important than others, and, least of all, how CagA benefits H. pylori for colonization of the stomach. For example, it has been proven that CagA is indeed injected into cells of the human gastric mucosa because gastric biopsy specimens from infected people contain the phosphorylated form of the protein. However, little is known about which cells are the targets of CagA injection and how this changes the life for colonizing bacteria. Given CagA’s connection with cancer, a particularly intriguing question is whether CagA is delivered to precursor cells in the mucosa, which could be affected in their differentiation pathways. Also important to explore is how CagA’s effects may alter the epithelium at different stages in the infection. For example, what are the effects of CagA in a healthy stomach compared with one in which the changes of atrophic gastritis have already altered the cellular composition of the glands? Many important answers await exploration in this regard.

One promising animal model to explore the pathogenic role of CagA in vivo appears to be the Mongolian gerbil. Mongolian gerbils have been shown to develop pathology similar to humans, and the TFSS and CagA contribute to the pathology in this model. As suggested by cell culture experiments, an important cellular pathway targeted by CagA in vivo are the epithelial junctions, in particular the E-cadherin/β-catenin pathway, which regulates epithelial cell adhesion, junction formation, and control of cell growth. In the gerbil model of H. pylori infection and carcinogenesis, CagA was found to activate the β-catenin pathway, causing its translocation into the nucleus. This effect was confirmed in the stomach of people harboring CagA+ strains.

In addition to the direct effects of CagA on the epithelial junctions, growth factor signaling, and the cytoskeleton, CagA, and the cag TFSS also have powerful proinflammatory effects. It is well documented that H. pylori infection elicits a strong inflammatory response and that this is accompanied by the expression of chemokines that recruit neutrophils, such as interleukin (IL)-8. It has also been shown that the CagA protein and the TFSS can both activate the nuclear factor (NF)-κB transcriptional response through independent mechanisms, leading to activation of proinflammatory signals and IL-8 secretion. In gerbils, its been shown that CagA mutants deficient in CagA or the function of the TFSS have much milder inflammatory changes, suggesting that much of the H. pylori-induced inflammation could be
secondary to the innate immune responses of the epithelium to the presence of CagA in the cytosol and the activation of nod1 by the T-FS. A recent intriguing finding is that the cag PAI may also trigger the accumulation of mutations through its activation of NF-κB. In cultured cells, it was shown that cag PAI+ strains of Helicobacter pylori induced the expression of a DNA-editing enzyme (AID), which resulted in the accumulation of mutations in the tumor suppressor p53.

In summary, CagA is a remarkable bacterial invention that has powerful effects on epithelial biology. However, despite all of these properties, most people receiving daily doses of CagA injected into the epithelium will not develop clinical evidence of disease, and, in those in whom this does occur, the effects of CagA will have accumulated over a very long period of infection, suggesting that we still need to understand the conditions under which CagA signals become pathogenic.

**Mechanisms of Persistence**

To colonize the human stomach for extended periods of time, *H. pylori* must overcome not just the physical and cellular barriers we have already discussed, but it also must avoid both the innate and the adaptive immune responses that are triggered in the stomach by its presence. We have already discussed the immunomodulatory effects of VacA and CagA and some of the *H. pylori* adhesins. Many other *H. pylori* products have been shown to have immunomodulatory effects. For example, although *H. pylori* urease is thought to function mainly as a protective buffering enzyme against gastric acidity, it also causes inflammation and cellular damage. HpNAP, a protein that may function as a bacterial ferritin or in DNA protection, has powerful neutrophil-activating activities and can induce the recruitment of polymorphonuclear leukocytes in vitro and in vivo. A number of *H. pylori* factors also appear to be designed to reduce inflammation or the recognition by the immune system. For example, *H. pylori* flagellar proteins have evolved to avoid being recognized by toll-like receptors. Also, the lipopolysaccharide (LPS) from *H. pylori* is 1000 times less pyrogenic and 500-fold less toxic than that of gram-negative enteric bacteria. This is likely due to modifications in the *H. pylori* LPS that may act as molecular mimics of human glycans to avoid immune recognition. For example, *H. pylori* possess several glucosyltransferase enzymes that have been implicated in LPS modification with carbohydrate groups resembling human Lewis blood group antigens Le(a), Le(b), Le(x), and Le(y). These modifications may also be important in pathogenesis through their contribution to autoimmunity.

As we have seen, *H. pylori* virulence factors elicit both proinflammatory and immunosuppressive effects. One hypothesis to explain this is that *H. pylori* induces a robust but specific form of chronic inflammation that is ineffective in clearing the infection, while avoiding forms of inflammation that would eliminate it. In agreement with this idea is the finding that, despite being largely an extracellular pathogen, *H. pylori* induces preferentially a T-helper cell 1 (Th1) type of T-cell response, classically thought of as a type of cell-mediated immunity essential for the control of intracellular pathogens. If a critical balance between inflammation and colonization is central to *H. pylori*’s survival, it is not surprising, as will be discussed below, that a number of human host polymorphisms lead to variations in the immune response and are critical in the development of gastric pathology. This concept has been explored over the past few years, and it is now apparent that host genetic factors interact with both bacterial virulence factors and environmental factors to define the ultimate clinical outcome. In the following section, we will discuss how these host genetic factors affect the ultimate clinical outcome and determine who develops a simple asymptomatic gastritis and who develops extreme outcomes such as gastric cancer and peptic ulcer disease.

**H. pylori Causes 3 Main Gastric Phenotypes That Determine Clinical Outcomes**

The basic process that mediates *H. pylori*-induced damage is gastritis with its associated humoral and cell-mediated immune mechanisms. It is now clear that the extent and distribution of this gastritis ultimately determine the clinical outcome. Three main gastric phenotypes have been identified, and each is associated with a set of pathophysiologic abnormalities that could explain why a certain outcome occurs (Figure 2). The commonest phenotype by far, which could be termed the “simple or benign gastritis” phenotype, is characterized by mild pan-gastritis with little disruption of gastric acid secretion. This phenotype is commonly seen in subjects who are asymptomatic and who on the whole develop no serious gastrointestinal (GI) disease. The second phenotype is the so-called duodenal ulcer phenotype and accounts for up to 15% of infected subjects, particularly in Western countries where peptic ulcers were common. This phenotype is characterized by an antral-predominant pattern of gastritis with relative sparing of the acid producing corpus mucosa. Subjects with this phenotype have high antral inflammatory scores, high gastrin, relatively healthy corpus mucosa, and very high acid output. These subjects also have defective inhibitory control of gastric acid secretion. This combination of pathophysiologic abnormalities contributes to the development of peptic ulcers, particularly duodenal and a large proportion of prepyloric ulcers. The third and most serious phenotype is the “gastric cancer phenotype,” which is characterized by a corpus-predominant pattern of gastritis, multifocal gastric atrophy, and hypo- or achlorhydria. These abnormalities, which affect approximately 1% of infected subjects, develop as a direct result of the chronic...
inflammation induced by the infection and increase the risk of gastric cancer. The gastric cancer phenotype is particularly prevalent in certain parts of Asia, where this cancer is common. Physiologically, the phenotype is characterized by low acid secretion, high gastrin, and low pepsinogen I and pepsinogen I/II ratio. The most intriguing aspect of this story is that subjects who develop duodenal ulcers are actually protected from developing gastric cancer, suggesting that the 2 outcomes are mutually exclusive.

It is clear, therefore, that \textit{H. pylori} infection can lead to several divergent clinical outcomes. Explaining this apparent paradox is essential for understanding the pathogenesis of \textit{H. pylori}-related disease in general and gastric cancer in particular. The last 2 decades have seen major advances in unraveling the contribution of bacterial virulence factors, environmental exposures, and host genetic factors in the pathogenesis of \textit{H. pylori}-induced diseases. In the following section, we discuss the role of host genetic factors in the pathogenesis of \textit{H. pylori}-induced clinical outcomes.

Role of Host Genetic Factors in \textit{H. pylori}-Induced Disease

As discussed above, \textit{H. pylori} causes its damage by initiating chronic inflammation in the gastric mucosa. This inflammation is mediated by an array of pro- and anti-inflammatory cytokines. Genetic polymorphisms directly influence interindividual variation in the magnitude of cytokine response, and this clearly contributes to an individual’s ultimate clinical outcome. In the case of \textit{H. pylori} infection, it is reasonable to speculate that the most relevant candidate genes would be ones whose products were involved in handling the \textit{H. pylori} exposure/attack (innate and adaptive immune responses) and ones that mediated the resulting inflammation. Because such a list of candidate genes would be prohibitively extensive, the initial search focused on genes that were most relevant to gastric physiology and, in particular, gastric acid secretion. As previously mentioned, \textit{H. pylori}-induced gastritis is associated with 3 main phenotypes that correlate closely with clinical outcome: duodenal ulcer phenotype, benign phenotype, and gastric cancer phenotype. Studies have shown that inhibition of gastric acid pharmacologically can lead to a shift from an antrum-predominant pattern (duodenal ulcer phenotype) to a corpus-predominant one with onset of gastric atrophy (gastric cancer phenotype). Thus, it was clear that an endogenous agent that was up-regulated in the presence of \textit{H. pylori}, has a profound proinflammatory effect, and was also an acid inhibitor that would be the most relevant host genetic factor to be studied. IL-1 \textunderscore \textit{B} fit this profile perfectly because, not only is it one of the earliest and most important proinflammatory cytokines in the context of \textit{H. pylori} infection, it is also the most powerful acid inhibitor known.

Role of IL-1 Gene Cluster Polymorphisms on \textit{H. pylori}-Induced Gastric Damage

El-Omar et al have shown that proinflammatory \textit{IL-1} gene cluster polymorphisms (\textit{IL-1B} encoding \textit{IL-1B} and \textit{IL-1RN} encoding its naturally occurring receptor antagonist) increase the risk of gastric cancer and its precursors in the presence of \textit{H. pylori}. Individuals with the \textit{IL-1B} \textunderscore 31\textsuperscript{C} or \textit{IL-1RN} \textsuperscript{*2} genotypes at increased risk of developing hypochlorhydria and gastric atrophy in response to \textit{H. pylori} infection. This risk also extends to gastric cancer itself with a 2- to 3-fold increased risk of malignancy compared with subjects who have the less proinflammatory genotypes.

Furthermore, the proinflammatory \textit{IL-1} genotypes increased the risk of both intestinal and diffuse types of gastric cancer, but the risk was restricted to the noncardia subsite. Indeed, the \textit{IL-1} markers had no effect on risk of cardia gastric adenocarcinoma, esophageal adenocar-
cinoma, or esophageal squamous cell carcinoma.\textsuperscript{135} The latter findings are entirely in keeping with the proposed mechanism for the effect of these polymorphisms in gastric cancer, namely reduction of gastric acid secretion. Thus, a high IL-1β genotype increases the risk of non-cardia gastric cancer, a disease characterized by hypochlorhydria, whereas it has no effect on cancers associated with high acid exposure such as esophageal adenocarcinoma and some cardia cancers. Interestingly, the high IL-1β proinflammatory genotypes do protect against the development of erosive and nonerosive esophagitis, suggesting again that they operate by reducing gastric acidity through induction of corpus atrophy.\textsuperscript{136,137}

The association between IL-1 gene cluster polymorphisms and gastric cancer and its precursors has been confirmed independently by other groups covering white, Asian, and Hispanic populations.\textsuperscript{138 -145} Machado et al\textsuperscript{138} were the first to confirm the association between IL-1 markers and gastric cancer in white populations and reported similar odds ratios to those reported by El-Omar et al.\textsuperscript{144} Furthermore, the same group subsequently reported on the combined effects of proinflammatory IL-1 genotypes and H pylori bacterial virulence factors (cagA positive, VacA s1, and VacA m1). They showed that, for each combination of bacterial/host genotype, the odds of having gastric carcinoma were greatest in those with both bacterial and host high-risk genotypes.\textsuperscript{139} This highlights the important interaction between host and bacterium in the pathogenesis of gastric cancer.

Unlike the studies mentioned above, some reports, particularly from Asian countries, failed to find an association between IL-1 markers and gastric cancer risk. A number of these studies were underpowered, whereas others used inappropriate controls. However, even excluding the weaker studies, there is still the impression that not all Asian or white populations have demonstrated a predisposition for gastric cancer in association with “published” proinflammatory IL-1 polymorphisms. In some instances, studies found that there was a positive association but with novel markers of the IL-1B gene.\textsuperscript{22} Other studies pointed to the importance of the background prevalence of gastric cancer in the population, with the positive associations being easier to demonstrate in low incidence compared with high incidence areas.\textsuperscript{144} Three meta-analyses have been published thus far to address the role of IL-1 markers in gastric cancer.\textsuperscript{146 -148}

Two of these concluded that the IL-1 proinflammatory genotypes increase the risk of gastric cancer.\textsuperscript{146,147} Taken at face value, these findings still point to IL-1β being a crucial cytokine in the pathogenesis of H pylori-induced gastric cancer and its precursors, and variations in its gene act as host genetic factors that mediate this effect.

A crucial piece of evidence that confirmed the unique role of IL-1β in H pylori-induced gastric carcinogenesis came from a transgenic mouse model in which IL-1β overproduction was targeted to the stomach by the H+/K+ ATPase β promoter.\textsuperscript{149} With overexpression of IL-1β confined to the stomach, these transgenic mice had a thickened gastric mucosa, produced lower amounts of gastric acid, and developed severe gastritis followed by atrophy, intestinal metaplasia, dysplasia, and adenocarcinoma. Crucially, these IL-1β transgenic mice proceeded through a multistage process that mimicked human gastric neoplasia. These changes occurred even in the absence of H pylori infection, which when introduced led to an acceleration of these abnormalities.\textsuperscript{150}

### Role of Other Cytokine Gene Polymorphisms

Soon after the IL-1 gene cluster polymorphisms were identified as risk factors for gastric cancer, the proinflammatory genotypes of tumor necrosis factor-α (TNF-α) and IL-10 were reported as independent additional risk factors for noncardia gastric cancer.\textsuperscript{135} TNF-α is another powerful proinflammatory cytokine that is produced in the gastric mucosa in response to H pylori infection. Like IL-1β, it has an acid inhibitory effect, albeit much weaker.\textsuperscript{151} The TNF-A-308 G>A polymorphism is known to be involved in a number of inflammatory conditions. Carriage of the proinflammatory A allele increased the odds ratio for noncardia gastric cancer to 2.2 (95% CI: 1.4–3.7). The role of the TNF-A-308 G>A polymorphism in gastric cancer was independently confirmed by a study from Machado et al.\textsuperscript{140} IL-10 is an anti-inflammatory cytokine that down-regulates IL-1β, TNF-α, interferon-γ, and other proinflammatory cytokines. Relative deficiency of IL-10 may result in a Th-1-driven hyperinflammatory response to H pylori with greater damage to the gastric mucosa. Homozygosity for the low-IL-10 ATA haplotype (based on 3 promoter polymorphisms at positions −952, −819, and −1082) increased the risk of noncardia gastric cancer with an odds ratio of 2.5 (95% CI: 1.1–5.7).\textsuperscript{135}

El-Omar et al studied the effect of having an increasing number of proinflammatory genotypes (IL-1B-511*T, IL-IRN*2*2, TNF-A-308*A, and IL-10 ATA/ATA) on the risk of nongastric cancer. The risk increased progressively so that presence of 3 or 4 of these polymorphisms increased the odds ratio for gastric cancer 27-fold.\textsuperscript{135} The fact that H pylori is a prerequisite for the association of these polymorphisms with malignancy demonstrates that, in this situation, inflammation is indeed driving carcinogenesis.

Another important cytokine that plays a central role in the pathogenesis of H pylori-induced diseases is IL-8. This chemokine belongs to the CXC family and is a potent chemoattractant for neutrophils and lymphocytes. It also has effects on cell proliferation, migration, and tumor angiogenesis. The gene has a well-established promoter polymorphism at position −251 (IL-8-251 T>A). The A allele is associated with increased production of IL-8 in H pylori-infected gastric mucosa.\textsuperscript{152} It was also found to
increase the risk of severe inflammation and precancerous gastric abnormalities in white\textsuperscript{152} and Asian populations.\textsuperscript{153} However, the same polymorphism was only found to increase risk of gastric cancer in some Asian populations\textsuperscript{153–156} with no apparent effect in white populations.\textsuperscript{157}

**Role of Polymorphisms in the Innate Immune Response Genes**

Genetic polymorphisms of inflammatory cytokines clearly play an important role in the risk of *H. pylori*-induced gastric adenocarcinoma. However, *H. pylori* is initially handled by receptors of the innate immune response, and it is conceivable that functionally relevant polymorphisms in genes of this arm of the immune system could affect the magnitude and subsequent direction of the host's response against the infection. The majority of *H. pylori* cells do not invade the gastric mucosa, but the inflammatory response against it is triggered through attachment of *H. pylori* to the gastric epithelium.\textsuperscript{86} Toll-like receptor 4 (TLR4), the LPS receptor, was initially identified as the potential signaling receptor for *H. pylori* on gastric epithelial cells.\textsuperscript{158} TLR4 belongs to a family of pattern recognition receptors, of which there are currently 11 members, that activate proinflammatory signaling pathways in response to microbes or pathogen-associated molecular patterns.\textsuperscript{159} TLR4, in conjunction with CD14 and MD-2, transduces signals through MyD88, Toll/IL-1 receptor domain, and TRAF6. This promotes transcription of genes, which are involved in immune activation including the transcription factor NF-κB and also mitogen-activated protein kinase pathways.\textsuperscript{160}

Arbour et al described a functional polymorphism at position +896 in exon 4 of the *TLR4* gene (dbSNP ID: rs4986790).\textsuperscript{161} This A>G transition results in replacement of a conserved aspartic acid residue with glycine at amino acid 299 (Asp299Gly) and alteration in the extracellular domain of the TLR4 receptor. This renders carriers hyporesponsive to LPS challenge by either disrupting transport of TLR4 to the cell membrane or by impairing ligand binding or protein interactions.\textsuperscript{161} The mutation has been associated with a variety of inflammatory and infectious conditions including atherosclerosis, myocardial infarction, inflammatory bowel disease, and septic shock.\textsuperscript{162–164} Recent work demonstrates that defective signaling through the TLR4 receptor ultimately leads to an exaggerated inflammatory response with severe tissue destruction, even though the initial immune response may be blunted. This is due to inadequate production of IL-10-secreting type 1 regulatory cells.\textsuperscript{165}

Hold et al\textsuperscript{166} recently hypothesized that the TLR4+896A>G polymorphism would be associated with an exaggerated and destructive chronic inflammatory phenotype in *H. pylori*-infected subjects. This phenotype would be characterized by gastric atrophy and hypochlorhydria, the hallmarks of subsequent increased risk of gastric cancer. In a recent study, Hold et al tested the effect of this polymorphism on the *H. pylori*-induced gastric phenotype and the risk of developing premalignant and malignant outcomes.\textsuperscript{166} The authors assessed associations with premalignant gastric changes in relatives of gastric cancer patients, including those with hypochlorhydria and gastric atrophy. Two independent white population-based case-control studies of upper GI tract cancer were also genotyped. TLR4+896G carriers had a 7.7-fold (95% CI: 1.6–37.6) increased odds ratio for hypochlorhydria; the polymorphism was not associated with gastric acid output in the absence of *H. pylori* infection. Carriers also had significantly more severe gastric atrophy and inflammation.\textsuperscript{166} Sixteen percent of gastric cancer patients in the initial study and 15% of the non-cardia gastric cancer patients in the replication study had 1 or 2 TLR4 variant alleles vs 8% of both control populations (combined OR, 2.4; 95% CI: 1.6–3.4).\textsuperscript{166} In contrast, prevalence of TLR4+896G was not significantly increased in esophageal squamous cell (2%; OR, 0.4) or adenocarcinoma (9%; OR, 0.8) or gastric cardia cancer (11%; OR, 1.2).

The association of TLR4+896A>G polymorphism with both gastric cancer and its precursor lesions implies that it is relevant to the entire multistage process of gastric carcinogenesis, which starts with *H. pylori* colonization of the gastric mucosa. Subjects with this polymorphism have an increased risk of severe inflammation and, subsequently, development of hypochlorhydria and gastric atrophy, which are regarded as the most important precancerous abnormalities. This severe inflammation is initiated by *H. pylori* infection, but it is entirely feasible that subsequent cocolonization of an achlorhydric stomach by a variety of other bacteria may sustain and enhance the microbial inflammatory stimulus and continue to drive the carcinogenic process. Evidence supporting this concept comes from the work of Sanduleanu et al, who showed that pharmacologic inhibition of acid secretion was associated with a higher prevalence of non-*H. pylori* bacteria.\textsuperscript{167} Furthermore, the simultaneous presence of *H. pylori* and non-*H. pylori* bacteria was associated with a markedly increased risk of atrophic gastritis and with higher circulating levels of IL-1β and IL-8. Supportive evidence\textsuperscript{168} also comes from animal studies in which hypochlorhydria was induced in mice either genetically (G−/G− gastrin-deficient mice) or pharmacologically (administration of omeprazole). Zavros et al\textsuperscript{168} found that genetic or chemical hypochlorhydria predisposes the stomach to bacterial overgrowth resulting in inflammation, which was not present in the wild-type mice or those not treated with an acid inhibitor. The composition of the gastric microbiota in health and disease is certainly complex, and more work is needed to appreciate fully the impact of host genetic factors.\textsuperscript{169}
The potential mechanism by which the TLR-4 polymorphism increases the risk of gastric cancer and its precursors is intriguing and may lie in the nature of the host’s overall response to the \textit{H pylori} LPS attack. Failure to handle the invasion by appropriately recognizing and activating the necessary pathways may lead to an imbalance of pro- and anti-inflammatory mediators. A very elegant demonstration of this phenomenon was recently reported by Higgins et al.\textsuperscript{165} The authors infected TLR4-defective C3H/HeJ mice and their wild-type counterparts (C3H/HeN) with an aerosol of \textit{Bordetella pertussis} (a gram-negative bacterium that causes whooping cough) and monitored the course of the infection and its consequences over several weeks. The course of the infection was more severe in the TLR4-defective than the wild-type mice, and this was associated with enhanced inflammatory cytokine production, cellular infiltration, and severe pathologic changes in the lungs. Most interestingly, Higgins et al showed that signaling through TLR4 in response to bacterial infection activated IL-10 production, which promoted IL-10-producing T cells and controlled inflammatory pathology during infection in normal but not TLR4-defective mice. It is therefore likely that the severe tissue damage observed in the TLR4-defective mice was due to the deficiency of the anti-inflammatory IL-10, which in turn accentuated the proinflammatory destructive tissue response.

We propose that subjects with an overall proinflammatory genetic makeup based on a combination of markers from the adaptive and innate immune systems (eg, IL-1\(\beta\), TNF-\(\alpha\), IL-10, IL-8, TLR4, mannose binding lectin\textsuperscript{179}) respond to \textit{H pylori} infection by creating an environment within the stomach that is chronically inflamed and with reduced acidity. This environment is conducive to the growth of other bacteria within the gastric milieu, leading to sustained inflammation and oxidative/genotoxic stress. Subjects with the same proinflammatory polymorphisms may respond in the same exaggerated manner to these non-\textit{H pylori} bacteria, thus maintaining the proneoplastic drive (Figure 3). This may explain why \textit{H pylori} is not required in the latter stages of gastric carcinogenesis and why it is often absent from gastric tumor tissue.

**Role of HLA Polymorphisms in Gastric Cancer**

Several studies have examined the role of HLA class I and II alleles in gastric cancer, in white and non-white populations. Lee et al found that the HLA class II allele DQB1*0301 was more common in white patients with gastric adenocarcinoma than noncancer controls.\textsuperscript{171} The mechanism linking HLA-DQB1*0301 with gastric adenocarcinoma was not clear but was thought to be independent of increased susceptibility to \textit{H pylori} infection. Azuma et al reported that the allele frequency of DQA1*0102 was significantly lower in the \textit{H pylori}-infected subjects with atrophic gastritis compared with infected subjects without atrophy and uninfected subjects.\textsuperscript{172} In addition, the allele frequency of DQA1*0102 was also significantly lower in the infected intestinal type gastric adenocarcinoma patients compared with all controls (infected and uninfected). They concluded that the HLA DQA1*0102 allele was protective against gastric atrophy and intestinal type gastric adenocarcinoma. Magnusson et al studied the effect of HLA class II alleles on risk of \textit{H pylori} infection and gastric cancer.\textsuperscript{173} Those authors confirmed Azuma et al’s finding that the DQA1*0102 allele was associated with decreased
risk of *H pylori* infection, but they found no protective effect on risk of gastric cancer. They also showed that the *DRB1* *1601* allele was significantly associated with an increased gastric cancer risk with an odds ratio of 8.7 (95% CI: 2.7–28.0). The effect of *1601* was more pronounced among *H pylori*-negative subjects, and the association was stronger with the diffuse, rather than with the intestinal, type of gastric cancer.56 Interestingly, Magnusson et al failed to show any association between gastric cancer and *H pylori*. The effect of increased gastric cancer risk with an odds ratio of 8.7

These 3 studies highlight several problems pertinent to the study of *H pylori* infection. The *HLA* system is highly polymorphic, and the genetic variation is very much dependent on ethnicity and its background selective heritage. As such, *HLA* disease associations in certain ethnic groups are unlikely to be relevant to others, simply because certain alleles may be under/overrepresented in these groups independent of the disease under question. The other relevant point here is the power required for such association studies. If one is dealing with the most highly polymorphic genetic system, it follows that the power of the studies has to match the complexity of this system. The study by Lee et al was based on 52 gastric cancer cases and 260 noncancer controls, whereas Azuma et al studied 82 cancer cases and 167 controls, and Magnusson et al had 130 cancer cases and 263 population controls. It could be argued that all 3 studies were grossly underpowered to look at associations between gastric cancer and *HLA* markers. Indeed, the sample size required will likely be in the thousands to get closer to any true associations.

**What is the Value of Identifying Host Genetic Factors Involved in *H pylori* Infection?**

Complex human diseases are often multifactorial, and their pathogenesis combines the effects of host and environmental factors. In the past, our research knowledge and technical know-how were limited in their ability to probe disease pathogenesis. The genetic revolution over the past decade has enabled scientists for the first time to examine afresh a multitude of unanswered clinical problems. Defining host genetic factors that control basic physiologic processes will explain many of the seemingly divergent phenotypic expressions of disease. Therefore, the most important benefit to the study of host genetics is the better understanding of disease pathogenesis. In the case of *H pylori* infection, host genetics has helped in confirming 2 very important facts: the first is the essential role of the initial insult in the form of the microbial challenge (in this case *H pylori*) and, second, is the important role of chronic inflammation with its long-term deleterious effects on gastric physiology. As such, the most sensible and practical conclusions from this knowledge are to either avoid getting the infection in the first place or to remove it or ameliorate its effects once it is established. The first is happening spontaneously, thanks to our improved hygiene and living conditions, but the second requires considerable thought and continued research. Although most of us would recognize the obvious theoretical benefit to ridding the world of *H pylori* in terms of preventing noncardia gastric cancer and peptic ulcer disease, there remains considerable doubt about what might happen to those in whom the natural history of this chronic infection has been disrupted. Does eradication work at all stages of the disease process, or is there a point of no return? Does the recovery of acid secretion lead to new problems? Is there an increased risk of acid-related cancer, eg, esophageal adenocarcinoma? These questions can only be answered through well-designed, adequately powered, and scientifically sound trials. Some of these trials are ongoing, and the next few years promise to clear up some of the controversies.

The other benefit to studying host genetic factors is in being able to predict clinical outcomes following certain exposures (microbial, chemical, dietary, pharmacologic, and others). For example, if we could define who might develop an atrophic, hypochlorhydric response to *H pylori* infection, this could form the basis for genetic screening so that these individuals could be offered eradication therapy. As things stand, this approach offers little benefit over simply checking for *H pylori* infection itself. The reason is that the currently identified genetic risk markers are very common in the population and are not specific enough to act as predictors of gastric cancer risk. It may be that future advances in affordable high-throughput genotyping could uncover a much more extensive genetic profile that satisfies the criteria for a screening test. If such a development were feasible, we must ensure that our governments enact laws that protect individuals from being discriminated against on the basis of their genetic heritage. These issues are not far-off coming, and the debate has to start now.

**Summary and Conclusions**

*H pylori* is the commonest chronic bacterial infection in the world, and, although asymptomatic in the majority of infected subjects, it is also the cause of significant human disease. Through the study of host-bacterial interactions in this simple model, significant advances have been achieved in our clinical GI practice. It is not an exaggeration to claim that the discovery of this bacterium and its acceptance by the scientific community created a major revolution that has already eradicated a major human illness (duodenal ulcer disease) and promises to eradicate a global lethal malignancy (gastric cancer). More importantly, the study of the bacterial virulence factors employed by *H pylori* and how these interact with hosts of different genetic backgrounds is redefining our understanding of bacterial ecology and homeostasis.
Because this bacterium is readily recoverable and its habitat is easily accessible and alterable, it should be regarded as perhaps the most informative of all microbial infections in biology. Twenty-five years after its discovery, we believe it is now only that we fully appreciate its full potential for enriching human knowledge.

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