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# VIRULENCE AND PATHOGENICITY OF *HELICOBACTER PYLORI*

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### *Helicobacter pylori*

may be the world's commonest chronic infection. Estimates suggest that more than half the world's population is infected by this gastric bacterium. Since the discovery and successful culture of *H. pylori* in the 1980s, it has become clear that the infection is important in the initiation of gastritis, resulting in numerous manifestations of gastroduodenal disease. Clinical outcomes associated with *H. pylori* infection include duodenal ulcer, gastric ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Specific genetic or phenotypic factors in infectious agents have been implicated as single causal factors in a variety of infectious diseases and associated outbreaks. It is well recognized that *Escherichia coli* 0157:H7 is causally associated with hemolytic uremic syndrome in the young and elderly. <sup>[104]</sup> Strains of two related cell lineages of *Streptococcus pyogenes*

account for nearly half of all cases of toxic shock-like syndrome, with almost all strains expressing the exotoxin A gene. <sup>[70]</sup> *H. pylori*

infection alone appears insufficient to explain fully the spectrum of diseases that are associated with chronic infection, however. Current data suggest that the pathogenicity of *H. pylori* depends on bacterial and host factors. Virulence of this infectious pathogen is based on factors that allow colonization and adaptation to the gastric environment and the stimulation of mediators of inflammation that contribute to the host physiologic and histologic changes. This article discusses bacterial and host immune factors important in gastroduodenal pathogenesis.

## BACTERIAL FACTORS IN PATHOGENESIS

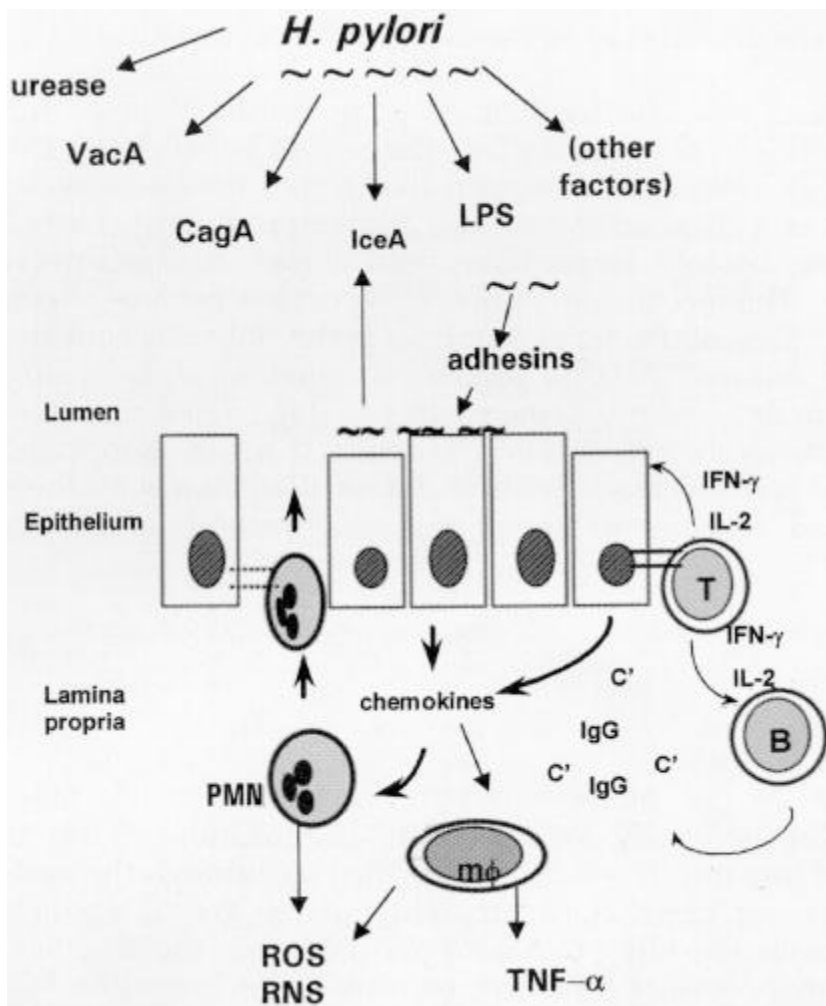
The bacteriology of *H. pylori*

has been discussed in detail in another article in this issue. Bacterial pathogenic or virulence factors can be grouped into those that are conserved among all strains of *H. pylori* for environmental adaptation and those that are differentially present and may be associated with different disease manifestations ([Table 1](#)). Conserved factors include factors such as urease, flagellins, and adhesins, which appear instrumental in host colonization. Nonconserved or variably present bacterial factors include candidate virulence factors comprising the genes for *cagA*, *vacA*, and *iceA* ([Fig. 1](#)). These latter genes have been speculated to affect the degree of inflammation and to influence disease presentation.

**TABLE 1 -- BACTERIAL AND HOST FACTORS IN GASTRODUODENAL PATHOGENESIS**

<i>H. pylori</i>	Host
Conserved	Cytokines
Urease	Growth factors
Flagellins	Lewis antigens
Adhesins	Class II MHC
Variable presence	Acid secretion
<i>cagA</i>	Mucus production
<i>cagA</i> PAI	Epithelial barrier
<i>vacA</i> alleles	
<i>iceA</i> variants	
Lewis antigens	

PAI = pathogenicity island; MHC = major histocompatibility complex.



**Figure 1.** Bacterial and host factors in the induction of mucosal inflammation and injury. *H. pylori* produces factors that allow colonization and promotion of tissue injury that ultimately result in a spectrum of gastroduodenal disease manifestations. Specific disease-promoting bacterial factors include urease, *cagA* gene, *vacA* subtypes, LPS, and possibly *iceA* variants. Adherence of bacteria to epithelial cells or release of soluble factors from *H. pylori*

induce a series of events in the epithelium leading to alteration of cell function and growth. Stimulation of expression of chemokines such as IL-8 initiates an inflammatory response, which involves phagocytes and mononuclear cells. The recruitment and activation of neutrophils results in the release of reactive oxygen species (ROS) and other mediators of injury. Macrophages serve as sources of ROS as well as reactive nitrogen species (RNS) and TNF-alpha that also contribute to damage of the host. Activated T cells, primarily of the Th1 phenotype, mediate injury through the release of IFN-gamma and potentially, through the generation of antibodies that form immune complexes that can also lead to tissue damage. How these pathways lead to the varying manifestations of *H. pylori* infection and how specific bacterial properties such as the presence of *cagA* affect these pathogenetic mechanisms remains to be established.

## CONSERVED HELICOBACTER PYLORI FACTORS

### Urease

#### *H. pylori*

urease plays a significant role in colonization and adaptation to the gastric acidic environment. Urease is important in the regulation of the microenvironment immediately surrounding it and for optimal survival of the microorganism in the gastric milieu. [79] [85] Eaton and Krakowa [26]

showed that urease was essential for the initial colonization of the normal gastric mucosa in gnotibiotic piglets. The role of urease may not have been solely to protect the organism from acid, however. These authors also found that the urease-deficient mutant could not colonize achlorhydric piglets with any reliability (<50%). [26] Preliminary data showed that clinical wild-type urease-negative *H. pylori* isolates also can colonize and infect an animal host, [89] emphasizing that factors in addition to urease may be essential for initial and chronic infection in the host.

The exact role and localization of urease in *H. pylori* has become controversial. *H. pylori* urease was thought to be

solely an extracellular protein. It is now clear, however, that active urease is present in the cytoplasm as well as on the surface and extracellularly. Early log phase bacteria and coccoid forms have been shown to contain cytoplasmic urease only. The mechanism of urease export has become of considerable interest. At present, bacterial autolysis is the only mechanism proposed for urease release. [77] [85]

This protein is being used as a single or in-combination antigen in vaccine trials. If a stage in the progression of the bacterial life cycle exists when *H. pylori* contain cytoplasmic urease exclusively, and this stage exists in mammalian stomachs, the success of urease-based vaccines in preventing experimental infection would be uncertain.

## Flagellin

Motility is one of the few *H. pylori*

characteristics that has been shown to be necessary for successful colonization of the gnotobiotic piglet. [27]

Presumably, *H. pylori*

must maneuver through the gastric mucus to adhere to or be in close proximity to the gastric epithelium. Two flagellin genes encoding *flaA* and *flaB* compose the flagellar filament, and both are necessary for active motility of the bacterium. [28]

## Adhesins

*H. pylori*

pathogenicity has been associated with active adherence of the bacterium to the gastric epithelium. Transmission electron micrographs have revealed that strains possessing the *cagA* gene and *vacA* s1 subtype often result in gastric microvilli effacement, pedestal formation, and actin condensation at the site of bacterial attachment. [86] Although *H. pylori* lipopolysaccharide (LPS) is less active than that of other bacterial species, Lewis antigens in *H. pylori* LPS have similarity to those in the gastric epithelium, and *H. pylori* LPS can express Le<sup>a</sup> and Le<sup>x</sup> determinants. [91] Expression of Lewis blood group antigens by *H. pylori*

LPS appears to mimic surface glycomolecules present in the gastric superficial and glandular epithelial regions, [67] which may help promote avoidance of host defense mechanisms and selective colonization. This mimicry also may allow further adaptation of the bacterium to specific sites in the gastric environment and may be important in host selection by different strains or in specific disease outcomes. [45]

## Cecropins

Cecropins are antibacterial peptides in *H. pylori*

that may be important to exclude other bacterial species from the gastric niche. [78] Derived from the ribosomal protein LI, these peptides showed antibacterial activity against gram-negative and gram-positive bacteria. *Altruistic autolysis* of *H. pylori* may allow release of these peptides into the gastric milieu to prevent colonization by other non-*Helicobacter* species.

## HELICOBACTER PYLORI GENOTYPES AND DISEASE ASSOCIATION

Virtually all individuals infected with *H. pylori*

exhibit chronic active gastritis, but only a proportion develop clinically significant disease manifestations, such as peptic ulcer or gastric cancer. Many infectious disease outbreaks can be traced back to a small number of strains or cell lineages within a bacterial species population. Whether this situation is true for *H. pylori*-related diseases remains confusing because of the large genetic heterogeneity in the *H. pylori* population species, the geographic variability in gastroduodenal diseases, and the recognition that genetically similar strains circulate within specific geographic regions. [16] [41] [63]

Identification of disease-related factors can be performed with various molecular epidemiologic techniques, such as restriction digestion analysis, ribotyping, arbitrary primer analysis, plasmid profiling, and single gene polymerase chain reaction (PCR), but these have been unsuccessful in identifying strains more important in the development of virulent disease. Despite the increasing number of *H. pylori* genes that have been cloned and characterized, only the *cagA*, *vacA*, and *iceA* genes have been identified as differentially present, suggesting disease specificity.

## **cagA Gene**

The *cagA* gene (cytotoxin-associated gene A) serves as the marker for the pathogenicity island (PAI). The *cag* PAI is an approximately 40 kb genomic region that contains 25 to 30 genes important for increased inflammation and for secretion of virulence-associated gene products. [1] [13] *H. pylori* strains with the complete *cag* PAI appear necessary for interleukin (IL)-8 induction, neutrophil recruitment, and tyrosine phosphorylation because these functions are absent when isogenic mutants of *cag*

PAI genes are studied. This genomic region includes sequence similarity to genes involved in transfer of macromolecules to target cells in *Bordetella* and *Agrobacterium*. Whether gene products from this region are important in secretion of *H. pylori* products is under investigation.

The expressed *cagA* gene product, CagA, is highly immunogenic. Many, but not all, clinical studies have shown that the CagA protein is associated with more severe clinical syndromes. Infection with *H. pylori* strains possessing the *cagA* gene causes increased gastric inflammation and greater risk of duodenal ulcer or gastric cancer. [13] [18] [20] Crabtree et al [20]

showed that host serum antibodies to the CagA protein were much more common, and higher titers were present in patients with duodenal ulcer compared with patients with nonulcer dyspepsia--100% in duodenal ulcer patients versus 63% in nonulcer dyspepsia patients (  $P=.001$ ). Covacci et al [16] showed that the *cagA* gene more commonly was present in strains isolated from duodenal ulcer patients compared with patients diagnosed with nonulcer dyspepsia--100% versus 75% (  $P=.0005$ ). Crabtree et al [20] showed that induction of IL-8 expression in gastric epithelial cells was associated with a CagA-positive phenotype. Warburton et al [102] studied patients from the United Kingdom with duodenal ulcer, gastric ulcer, or nonulcer dyspepsia; they found *cagA*-positive strains were significantly commoner in patients with duodenal ulcer compared with patients with nonulcer dyspepsia (94% versus 56%). Takata et al [90] found *H. pylori* expression of CagA significantly greater in Japanese patients with duodenal ulcer compared with nonulcer dyspepsia patients--98% versus 61% (  $P<.001$ ).

In contrast to the aforementioned data, some studies suggest that the *cagA* gene is common in *H. pylori*-infected subjects from developing countries regardless of their clinical presentation. In contrast to Takata's study, Matsukura et al [60] found no difference in the frequency of the *cagA* gene in Japanese patients with gastritis (74%), duodenal ulcer (80%), gastric ulcer (82%), or gastric cancer (77%). In 171 Taiwanese patients, 100% of those with peptic ulcer disease or gastric cancer and 94% of those with nonulcer dyspepsia were infected with a *cagA*-positive *H. pylori* strain. [108] When serum CagA antibodies were compared in peptic ulcer patients with healthy asymptomatic volunteers from a single center in Houston, Texas, neither CagA antibody presence nor titers allowed discrimination between patients with peptic ulcer or subjects with asymptomatic gastritis. [44] Likewise, Mitchell et al [65] found no association in serum CagA antibodies in Chinese patients with various gastroduodenal diseases diagnosed by endoscopy.

## **cagA in Duodenal Ulcer and Gastric Cancer: A Paradox**

The *cagA* gene is associated strongly with duodenal ulcer and gastric cancer. These two diseases are almost mutually exclusive, however. If this single gene is significant in these two vastly different clinical outcomes, heterogeneity within the *cagA* gene might be important. Four different primer sets from published studies were used to determine accuracy of PCR detection of the *cagA* gene in a sample of isolates obtained from patients from various geographic regions. [56] This study showed that apparent absence of the *cagA* gene using a single PCR may be related to lack of specificity of those PCR primers; this is not surprising given the large genetic heterogeneity of the *H. pylori* population. Both 5' and 3' regions of the *cag*

A gene in Korean isolates were highly different from isolates from patients in the Western Hemisphere. This situation confirms that genetic detection techniques for *H. pylori* studies must be validated within a population and confirmed in other geographic regions. This study also showed a lack of association between *cagA* gene presence and specific disease presentation.

The *cagA* gene indicates the presence of the *cag*

PAI, which was probably acquired from a foreign bacterial species by horizontal transfer. [13] This approximately 40 kb region containing 25 to 30 genes has been associated with enhanced inflammation and signal transduction in the host. Jenks et al [50] attempted to show association of *cag* PAI genetic patterns with specific disease in isolates from an Italian population. The PCR amplification of nine different regions of the PAI did not show a pattern association with specific disease outcome. The frequent presence of the *cagA* gene in *H. pylori* isolates from patients independent of disease presentation suggests that the *cag* PAI may represent a nonspecific genetic virulence region.

## ***vacA* Gene**

The *vacA* gene (vacuolating cytotoxin A) is present in almost all *H. pylori* strains but is expressed in only about half. [93] Expression of the VacA protein is associated with vacuolation of many eukaryotic cell lines through alterations in intracellular membrane fusion. [17] Different alleles have been classified in the 5' signal region (s-region) and the middle region (m-region) of the *vacA* gene. [3] The s-region is present as *s1* (which can be distinguished further as *s1a*, *s1b*, *s1c*) or *s2*, whereas the m-region is present as *m1* or *m2*. Production of the vacuolating cytotoxin VacA is designated by the allelic combination *s1/m1* and *s1/m2*. In many studies, *H. pylori* isolates with the *s1/m1* combination produce larger amounts of the toxin than strains with other combinations and are isolated more frequently from duodenal ulcer patients. Early histologic studies have shown an increased epithelial cell vacuolation and inflammation in patients infected with strains expressing the VacA protein. [4] Basso et al [8] showed a significant correlation of *H. pylori cagA* and *vacA s1* in Italian patients with gastric adenocarcinoma and peptic ulcer disease compared with patients with *H. pylori* gastritis only.

## ***iceA* Gene**

The *iceA* (induced by contact with epithelium) gene has a genetic structure similar to a restriction endonuclease. [76] Allelic variants have been categorized as *iceA1* or *iceA2* based on heterogeneity within the gene. [76] [98] *iceA1* has been suggested as a marker for enhanced predisposition to peptic ulcer disease because of its more frequent presence in peptic ulcer patients from some geographic regions. *iceA1* may be identified more frequently in strains from peptic ulcer patients, but its presence seems not to be specific for duodenal versus gastric ulcer disease. The role of this gene in clinical disease manifestations has not been studied extensively, and association with specific disease remains uncertain.

## **GEOGRAPHIC DISTRIBUTION OF *HELICOBACTER PYLORI* STRAINS**

Miehlke et al [63]

reported that 98% of isolates from Korea showed positive PCR results with a primer set targeting the middle region of the *cagA* gene, but only 1.7% of the same set of isolates had positive results with another primer set assessing a region near the 3' end. These PCR patterns were not observed in isolates from Houston, however, suggesting that distinct *H. pylori* populations may circulate in different geographic regions. Similar results were observed in two other studies. [74] [96] Amplification for *cagA* with one set of primers yielded positive PCR results in only 52% of Chinese *H. pylori* isolates, which were characterized as *cagA* positive by Southern or colony hybridization. PCR using the same primer set detected 92% of the *cagA*-positive Dutch isolates, which had already been determined as *cagA* positive by hybridization methods. These results indicated allelic variation in the *cagA* gene with different genotypes of *H. pylori* circulating in China and the Netherlands.

As with the *cagA* gene, discrepancies among published studies examining *vacA* subtypes and association with specific gastrointestinal disease are present, particularly in populations from developed versus developing countries. Takata et al [90]

detected a statistical difference in CagA between patients presenting with peptic ulcer compared with patients with nonulcer dyspepsia. They found no difference in the distribution of VacA in these Japanese patient groups, however. [90]

Warburton et al [102]

had similar findings in a sample of patients from the United Kingdom who presented with active or history of duodenal ulcer, gastric ulcer, or nonulcer dyspepsia. They found a significant association between *H. pylori cagA* and peptic ulcer disease, but the *vacA s1* subtype was detected in 89% of the sample regardless of gastroduodenal disease type. [102]

One study compared *vacA* genotype in *H. pylori*

isolates from patients from the United States, Korea, and Colombia. No significant differences were seen in the *vacA* signal or midgene regions when comparing patients presenting with duodenal ulcer, histologic gastritis, or gastric cancer. [40] Both *vacA s1a* and *s1b* allelic variants were common in the United States and Colombia, but *vacA s1a* was more common in strains from Korean patients, independent of disease presentation. Findings related to *vacA* genotype and disease outcome in various geographic studies suggested that neither *vacA* signal nor midgene region was predictive of disease outcome. [107] Geographic distribution of *vacA* allelic types in 24 countries was investigated by van Doorn et al. [97] They confirmed the common presence of *vacA s1a* and *s1b* in Europe and North America; *vacA s1b* was detected in most strains from South America, whereas the *vacA s1a* was the predominant *vacA* signal variant in

Asia. These findings support the concept that different genetically related strains circulate within geographic regions, and disease association of *H. pylori* genetic factors may be a reflection of the *H. pylori* population in that particular region. [16]

## HELICOBACTER PYLORI AND GASTROESOPHAGEAL REFLUX DISEASE

Whether *H. pylori*

markers influence clinical outcome in esophageal disease has become the subject of clinical investigations. One study found no significant difference in prevalence of *H. pylori* in controls compared with patients with various esophageal disorders, [100]

but other preliminary studies suggest that some patient populations with esophageal disease have less frequent *H. pylori* infection. The above-mentioned study suggested that although *H. pylori* prevalence was not different between controls and patients with a variety of esophageal disorders (e.g., esophagitis, Barrett's esophagus, dysplasia or adenocarcinoma), the presence of the *H. pylori*

*cagA* was significantly less in strains from patients with advanced or more severe esophageal disease. The investigators proposed that *H. pylori* with the *cagA* gene might lend a protective effect against severe esophageal disease. The lower frequency of *cagA*-positive strains in patients with esophageal dysplasia and cancer needs to be confirmed in large series to establish whether a significant relationship exists.

Labenz et al [54] suggested that eradication of *H. pylori* in duodenal ulcer patients provoked a 25% increase in reflux esophagitis during a 2-year follow-up. Subsequent correspondence from the authors indicated that only 3% of patients developed symptoms over the follow-up period. Rather than new-onset gastroesophageal reflux disease and esophagitis, this could represent the unmasking of gastroesophageal reflux disease by elimination of peptic symptoms. An endoscopic follow-up study in duodenal ulcer patients in the United States revealed a decrease in gastroesophageal reflux disease symptoms during the 6-month posttreatment period with no increase in esophagitis. [94] Another preliminary report of a post- *H. pylori*

eradication study in dyspeptic patients suggested that heartburn decreased significantly in the first year of follow-up and did not change during the second follow-up year. [14]

The final verdict awaits the conclusion of the numerous studies in progress.

Most studies have focused on these genes as single markers of disease manifestations, but because of the conflicting results from different populations, using these genetic factors as predictive or risk factors should be done with caution. For a factor to be significant in specific disease manifestation, it must be present more commonly in strains from all geographic regions, not just in certain populations. Accuracy of the technique used to establish presence or heterogeneity of the gene must be confirmed by a second method. Comparison among studies or isolates must use the same product (i.e., studies of the *cagA* gene should not be compared with those examining titer or presence of antibodies to the CagA protein because these are different methodologies).

The cumulative presence of these markers may be much more helpful in assessing predisposition for clinical presentation. One study from van Doorn et al [98] suggested that the combination of *cagA* and *vacA* s1/m1 and *iceA1* may allow prediction of the person more likely to present with peptic ulcer disease. These studies have been performed only in small European populations and must be confirmed in populations throughout the world. The role of these bacterial factors in disease presentation is also under active investigation using animal models of *H. pylori*-related disease.

## HELICOBACTER PYLORI AND NONGASTROINTESTINAL HOST DISEASE

Although *H. pylori*

has been detected commonly in large samples of patients with various systemic disorders, including coronary artery disease, rosacea, autoimmune disease, short stature, migraine headaches, and allergic rhinitis, no causal role between the infection and such disorders has been identified. [55]

## HOST FACTORS IN PATHOGENESIS

Increasing worldwide investigations have begun clarifying the host factors in *H. pylori* infection and associated diseases. [39]

Details of the host immune response are crucial to understand the bacterium's ability to evade host defense mechanisms and the role of *H. pylori* in cellular proliferation and apoptosis. [30]

### Interactions With Gastric Epithelial Cells

It is apparent that epithelial cells play an integral part in the host response to *H. pylori* infection as well as being the target of infection. *H. pylori* are regarded as noninvasive organisms, yet they induce a significant inflammatory response that is orchestrated through their interaction with the epithelium. Epithelial cells express molecules that serve as receptors for *H. pylori*, including Lewis antigens [45] and class II major histocompatibility complex (MHC) antigens. [31] Activation of epithelial cells by *H. pylori*, through binding or possibly by soluble bacterial factors, results in alterations of cell function and phenotype through signaling mechanisms that are just beginning to be understood. Infection induces the expression of cytokines and growth factors as well as cell surface proteins that mediate interactions with other cell types. Direct effects of infection and those resulting from activation of immune cells lead to alterations of epithelial growth and differentiation as well as cell death.

### Cytokines and Growth Factors

#### *H. pylori*

infection is associated with increased mucosal levels of IL-1, IL-6, IL-8, and tumor necrosis factor (TNF)-alpha. The neutrophil-activating and chemoattractant chemokine, IL-8, is produced by epithelial cells as well as other cells within the gastric mucosa. Increased epithelial staining for IL-8 has been shown in gastric biopsy specimens from *H. pylori*-infected patients compared with uninfected subjects. [21] In vitro studies have shown enhanced IL-8 mRNA and protein expression after *H. pylori* infection in gastric epithelial cell lines. [19] [23] [48] [88] This expression depends on the adherence of live organisms [23] [88] and is increased by strains with the *cagA*-positive phenotype, [19] [88] although *cagA*-negative mutants are still able to induce IL-8. [22] [88] Total absence of the *cag* PAI, however, fails to induce IL-8 expression. [88]

Stimulation of IL-8 involves signaling through NF-kappaB [87] and activator protein (AP)-1 and tyrosine phosphorylation. [10]

Other members of the chemokine family of cytokines, including Gro-alpha as well as MCP-1, RANTES, and other members of the C-C family of chemokines, are increased by *H. pylori*. [38] [52] Because C-C chemokines exert their actions on cells other than neutrophils, these findings substantiate a role for the epithelium in initiating the inflammatory response with lymphocytes, monocytes, and eosinophils as well as the better-established epithelial relationship with neutrophils.

The effect of growth factors and their receptors, which mediate epithelial cell growth and repair, also are altered by infection. *H. pylori*

infection stimulates the expression of epidermal growth factor-related peptides but inhibits their proliferative effect in vitro. [81]

Epidermal growth factor-receptor synthesis and phosphorylation are inhibited by vacuolating cytotoxin and culture supernatants from *vacA*-positive strains. [73] The effect of *H. pylori* infection and the associated inflammatory response on cell cycle events is an area that is just beginning to be investigated and is related to understanding of how infection leads to metaplasia, dysplasia, and gastric cancer.

### Cell Surface Molecules

Gut epithelial cells express many molecules that are involved in interactions with neutrophils and lymphocytes. Class II MHC molecules are involved in the presentation of exogenous antigen to CD4<sup>+</sup> T lymphocytes. Many cell types express class II MHC molecules, including B cells, endothelial cells, and a variety of epithelia. Similar to intestinal epithelial cells, gastric epithelial cells have been shown to express class II MHC in native tissues as well as in cultured cells. [15] [29] [83] [84] [95]

Increased epithelial class II MHC expression has been reported in gastric biopsy specimens from patients with gastritis. [29] It is not clear that infection stimulates class II MHC expression directly, but cytokines known to be increased during

*H. pylori* infection, [53] [69] [72] [106] such as interferon (IFN)-gamma and TNF-alpha, have this effect. [83] Invariant chain (Ii), a molecule that regulates class II MHC function, also is expressed by gastric epithelial cells, and this expression is increased in cell lines as well as native tissues during *H. pylori* infection. Studies show that *H. pylori* binds to class II MHC molecules to stimulate programmed epithelial cell death [31] and that urease may be one bacterial protein that mediates this event. [33]

At least two signals from antigen-presenting cells are required to activate T cells. The first signal is antigen specific and occurs after T-cell receptor recognition of the MHC-antigen complex. The second or *costimulatory* signal is generated through the interaction of receptors and coreceptors on antigen-presenting cells and T cells. Full T-cell activation and effector function depend on the interaction of the costimulatory molecules B7.1 (CD80) or B7.2 (CD86) with CD28 on T cells. [11] [47]

Although B7 expression has not been well substantiated in intestinal epithelial cells, B7 has been shown in isolated human gastric epithelial cells as well as gastric epithelial cell lines. [109] These studies showed that B7 expression is increased in isolated gastric epithelial cells from patients with *H. pylori* infection compared with uninfected controls.

Other immune adhesion molecules, including ICAM-1 (CD54), the counterreceptor for the beta-integrin molecules found on neutrophils and T lymphocytes, and lymphocyte function antigen (LFA)-3, are expressed by gastric epithelial cells to regulate immune interactions during *H. pylori*

infection. It is now recognized that the gastric epithelium can express Lewis antigens that have similarity to those found in *H. pylori* LPS. [67]

Lewis antigens on the epithelium act as adhesins that mediate bacterial attachment and may induce autoimmune gastritis through the formation of antibodies to host proteins. Transgenic mice expressing Lewis b in epithelial cells have been shown to have more severe gastritis with anti-parietal cell antibodies and atrophy than the Lewis b negative control animals. [45]

## Alteration of Cell Function

Acid secretion is a major function of the gastric epithelium that is regulated by a variety of neural, endocrine, and immune factors. Elevated fasting and meal-stimulated or hormone-stimulated levels of gastrin are well documented in *H. pylori* infection, and there is evidence that gastrin expression is regulated by bacterial factors and cytokines. [9]

Expression of somatostatin, an acid-inhibitory peptide, is diminished in infected individuals, as is duodenal bicarbonate secretion. The net effect of *H. pylori*

infection on acid secretion is complex and varies depending on the presence of duodenal ulcer disease, duration of infection, and presence of mucosal atrophy. This topic is discussed in more detail in another article in this issue.

Secretion of mucus is another important function of the gastric epithelium that is affected by *H. pylori* infection. In vitro studies employing an intestinal epithelial cell line showed a small decrease in basal mucus secretion 24 hours after *H. pylori* infection, whereas responses to secretory agonists were strongly inhibited. [62] Gastric mucosal hydrophobicity is reduced in *H. pylori*-infected individuals, with reversal of this abnormality after eradication of infection, [42] [43] [57] although it has been suggested that this may be an in vitro artifact. [59]

Epithelial barrier function is altered during *H. pylori*

infection. In a preliminary study conducted in primary rabbit gastric epithelial cells cultured on membranes with 3- $\mu$ m pores, IL-8-stimulated transmigration of neutrophils was associated with increased back-diffusion of sodium ion, suggesting some modification of gastric epithelial permeability. [36] In another preliminary study, *H. pylori* infection of a polarized intestinal epithelial cell line was accompanied by an increase in transcellular macromolecular transport. [61]

Sonicates of *H. pylori* decreased transepithelial resistance and increased paracellular permeability in epithelial monolayers, an effect that was inhibited by protein kinase C activators. [92] In human tissues, *H. pylori* infection results in abnormal localization of tight junction proteins, occludin and zona occludins-1 (SH Phadnis et al: personal communication). These studies indicate that alterations of barrier function, similar to many other host responses to infection, are a consequence of direct effects of *H. pylori* and the accompanying inflammatory response.

## Alteration of Cell Growth

Proliferation of the gastric epithelium has been shown to be altered in gastric tissues and cell lines, although the results differ markedly in these two groups. In general, studies of biopsy samples obtained from *H. pylori*-infected subjects indicate that gastric epithelial proliferation is increased, with a return to normal growth after eradication of infection. [12] In contrast, most studies with cell lines show an inhibitory effect of *H. pylori* on epithelial cell growth with delayed restitution. [32]

Direct effects of the bacteria as well as secreted products have been proposed to alter epithelial growth during *H. pylori* infection. A bacterial factor unrelated to *cagA* or *vacA* has been reported that inhibited gastric epithelial growth. [101] In other in vitro studies,  $\text{NH}_2\text{Cl}$  and  $\text{NH}_4\text{Cl}$  inhibit rat mucosal growth and epithelial cell migration. [25] [71]

It is likely that factors released from other cell types within the gastric mucosa play a role and may explain differences in proliferation found in tissue samples and in cultured cells. Potential stimulatory factors include certain cytokines and growth factors, although others may reduce growth. There is some evidence that differentiation of epithelial cells may be altered by *H. pylori*

infection because changes in the numbers of parietal, chief, and mucous neck cells have been reported in *Helicobacter*-infected mouse models. [35]

One can speculate that the balance of inhibitory or stimulatory influences on epithelial growth and differentiation may mediate epithelial outcomes, including ulceration, intestinal metaplasia, and gastric neoplasia.

Cell death represents another mechanism whereby epithelial growth can be regulated. In contrast to necrosis, apoptosis is a programmed form of cell death in which accumulation of DNA fragments consisting of multiples of 180 bp forms the basis of several assays to detect apoptosis. Defective apoptosis plays a role in carcinogenesis, and excessive apoptosis may lead to ulceration. Apoptosis rarely is detected in normal intestinal epithelium but is increased in melanosis coli, in graft-versus-host disease, in nonsteroidal anti-inflammatory drug-induced enteropathy, human immunodeficiency virus infection, and with chemotherapy or radiation. [103] Apoptosis had not been as well studied in the stomach, but it is now clear that *H. pylori* infection is associated with increased apoptosis.

Moss et al [68]

showed that apoptotic cells were rare in uninfected gastric tissue samples, with a mean of 2.9% of epithelial cells, located in the most superficial aspect of the gastric glands. In infected tissues, apoptotic cells were located throughout the depth of the gastric glands and increased in mean numbers (16.8%), a value that fell to 3.1% after *H. pylori* eradication. Other studies have confirmed these findings, [51] [58] and it has been suggested that increased epithelial apoptosis may be due to *cagA*-negative strains because Peek et al [75] showed increased apoptosis in biopsy specimens from patients infected with *cagA*-negative strains. *cagA*-positive strains were associated with increased epithelial proliferation, but rates of apoptosis were similar to those in uninfected controls. Other reports have failed to confirm this association, and in vitro studies indicate that *cagA*-positive and *cagA*-negative *H. pylori* strains can induce apoptosis, [101] although there is some evidence that bacteria lacking the *cag* PAI have a lesser effect. [64] It does not appear that the *cagA* status of the bacteria alone can predict the outcome of infection in vitro, and other factors must be involved to develop the varying manifestations of *H. pylori* infection.

Several mechanisms have been proposed to explain the direct effects of bacteria on programmed gastric epithelial cell death, including Fas-Fas ligand interactions, signaling through class II MHC, [31] and the generation of reactive oxygen species (ROS) [5] [80] and reactive nitrogen species (RNS). [105] A role for RNS is suggested by a study showing increased immunohistochemical staining for inducible nitric oxide synthase expression and for a marker of peroxynitrite formation in *H. pylori*-infected gastric epithelium. [58] Inflammatory cell products (discussed subsequently), including cytokines such as TNF-alpha and interferon-gamma (IFN-gamma), [31] [101] as well as ROS and RNS, [37] also can induce apoptosis.

## Interactions With Immune Cells

Infection with *H. pylori*

results in a unique inflammatory response in which infection persists despite the recruitment and activation of T and B lymphocytes, phagocytic cells, and other immune cell populations. The roles of eosinophils and mast cells, which are increased in the gastric mucosa during *H. pylori* infection, are not well understood. Lymphocytes and neutrophils play a major part in the host response to infection and are discussed subsequently.

## Lymphocytes

The accumulation of T cells in the gastric mucosa of *H. pylori*-infected patients reflects recruitment and activation of T cells during infection. Two subsets of T helper cells, referred to as *Th1* and *Th2* cells, can be distinguished by the pattern of cytokine expression. *Th1* cells mediate cellular immune responses to malignancy and intracellular infection, whereas *Th2* cells typically are involved in secretory immune responses of mucosal surfaces and allergic reactions. Although one would expect a *Th2* response in this noninvasive mucosal infection, there is good evidence that the *Th1* subset of T cells predominates in *H. pylori* infection, selecting for a cell-mediated type of immunity through the production of IFN-gamma and IL-2. Freshly isolated

T cells from *H. pylori*-infected mucosal biopsy specimens produced Th1 cytokines and low-to-absent levels of Th2 cytokines, IL-4 and IL-5. [7] Bacteria themselves may select for the Th1 phenotype because live *H. pylori* stimulate IL-12, a cytokine that acts to select Th1 cells. [46]

The Th1-predominant immune response may lead to T cell-mediated epithelial damage mediated by IFN-gamma and through Fas-FasL interactions. An imbalance between Th1 and Th2 may lead to generation of autoimmune processes.

Antibodies directed to *H. pylori*

have been reported that cross-react with the gastric epithelium and may induce damage through immune complex-mediated injury to the epithelium. [67]

It is apparent that Th1 response is potentially deleterious to the host, and there is some evidence to suggest that selecting for Th2 responses has a beneficial effect on the outcome of infection. In a murine model of *H. pylori* infection, transfer of Th2 cells from infected mice into infected recipients reduced bacterial load, whereas transfer of Th1 cells increased the degree of gastritis. [66] The immunology of *H. pylori* infection and prospects for vaccines are discussed further in another article in this issue.

## Phagocytic Cells

Infection induces epithelial expression of a panel of chemokines, including IL-8, which recruit and activate neutrophils, resulting in the *active* component of the chronic active gastritis that characterizes *H. pylori* infection. This aspect of the inflammatory response does not appear to be effective in eliminating infection, although phagocytic cells can be found ingesting *H. pylori*

organisms. Instead, neutrophils and macrophages contribute to damage to the host through the generation of inflammatory mediators, including eicosanoids, ROS, and RNS, as well as cytokines, particularly TNF-alpha. TNF-alpha plays an important part in the host response to infection because it regulates acid secretion and epithelial cytokine induction and may mediate cell death.

ROS, including superoxide ( $O_2^-$ ), hydroxyl radical ( $OH^\bullet$ ), and hydrogen peroxide ( $H_2O_2$ ), are generated during the respiratory burst in phagocytic cells through mitochondrial and microsomal electron transport chains and by oxidant enzymes, including xanthine oxidase, cyclooxygenase, and lipoxygenase. In the presence of neutrophil myeloperoxidase,  $H_2O_2$

combines with free chloride to form hypochlorous acid (HOCl). HOCl is approximately 100 times more reactive than  $H_2O_2$ , can react with primary amines to form *N*-chloramines and monochloramine, and eventually leads to lipid damage.

[49] Hydroxyl radicals are extremely reactive and short lived. Neutrophils and macrophages also express NO synthase and produce NO. NO

has been shown to inhibit mitochondrial enzymes, such as aconitase, and inhibit the electron transport chain. NO toxicity may be caused directly by the inhibition of normal mitochondrial function and the leakage of reactive oxygen. NO can react with superoxide to form peroxynitrite ( $ONOO^-$ ), which can break down spontaneously to form hydroxyl radical or singlet oxygen, both of which are highly reactive with DNA.

The three major types of cellular damage resulting from ROS are lipid peroxidation, protein oxidation, and oxidation of DNA. ROS, such as  $OH^\bullet$

, are thought to play a causal role in malignant transformation through the induction of DNA damage, which comprises single strand breaks and alkali-labile sites in DNA as well as many forms of base damage. A critical cell cycle control protein, p53, which is induced after DNA damage and is important for DNA damage-induced apoptosis, directs cellular arrest in  $G_1$  by the induction of several proteins, such as p21/WAF-1. [49] This delay in cell cycle progression allows the cell to repair the damaged DNA. If the damage is severe, however, the cell undergoes a p53-dependent programmed cell death. Oxidative DNA damage is increased in gastric epithelial cells by *H. pylori* infection, [6] [34] with epithelial and phagocytic cells and possibly bacteria themselves serving as sources of ROS. [24] [80] Because infection is associated with decreased levels of a tissue antioxidant scavenger, vitamin C, [82] and because there is evidence that antioxidants may ameliorate the development of premalignant epithelial changes in *H. pylori* infection, there is growing interest in understanding the role of oxidative stress in infection. [99]

## SUMMARY

Bacterial and host response factors play significant roles in the pathogenicity of *H. pylori*-related disease manifestations.

The complete DNA sequences for two *H. pylori* strain genomes have been published. [2] The differences in the sequences between these two unrelated strains may enable clinicians to identify rapidly other conserved and potentially virulent genes and products. Whether these two DNA sequences are sufficient representation of the *H. pylori* genetic heterogeneity is unknown. The host immune response and the cascade of events that occurs with *H. pylori* infection are being clarified rapidly. Understanding the role of this gastric bacterium in apoptosis and cellular proliferation would enable clinicians to understand its relationship to ulcerogenesis and gastric malignancy. Piecing together many observations related to *H. pylori* would result in understanding the interaction of *H. pylori* factors and host responses that lead to the variety of disease manifestations associated with this chronic infection. The development of animal models with *H. pylori* and other *Helicobacter* species has set the stage in which in vitro observations can be tested in the in vivo model.

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