Events at the Host-Microbial Interface of the Gastrointestinal Tract

IV. The pathogenesis of Helicobacter pylori persistence

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The pathogenesis of Helicobacter pylori persistence. Am J Physiol Gastrointest Liver Physiol 289: G8–G12, 2005; doi:10.1152/ajpgi.00086.2005.—Long-term interactions between Helicobacter pylori and humans significantly increase the risk for peptic ulcer disease and noncardia gastric adenocarcinoma. The vast majority of infected persons remain persistently colonized unless a targeted antibiotic regimen is employed; thus regulation of inflammation by H. pylori is governed by levels of host-bacteria equilibria that are not found during cellular interactions with acute enteric pathogens. It is important to gain insight into mechanisms that regulate immune evasion by H. pylori not only to develop more effective treatments for disease, but also because such knowledge may serve as a paradigm for the role that other chronic infectious agents play in the genesis of pathological lesions that arise from inflammatory foci.

gastric cancer; ulcer; inflammation

A signature feature of the gastric inflammatory response to Helicobacter pylori is the capacity to persist for decades. This is in marked contrast to inflammatory reactions induced by other mucosal pathogens, such as Salmonella, that either resolve within days or progress to eliminate the host. Coevolution of H. pylori with humans over thousands of years has refined the interactions that occur between bacterial and host effectors. However, biological costs are incurred by these long-term relationships in that chronic colonization confers an increased risk of developing peptic ulceration, gastric adenocarcinoma, and non-Hodgkin lymphoma of the stomach (18). Many H. pylori constituents previously classified as being essential for initial colonization or virulence also have the capacity to manipulate the host immune response, which permits long-term inhabitation of the stomach. This review will focus on specific molecular interactions that facilitate H. pylori persistence and pathogenesis, processes that are inextricably linked and that directly affect disease outcomes associated with this organism.

INITIAL OCCUPATION OF THE GASTRIC NICHE

Gastric acidity and peristalsis normally preclude bacterial colonization of the human stomach, but natural selection has provided H. pylori with several mechanisms to elude these primary defenses and establish persistent infection. One pH-altering mechanism used by H. pylori is production of urease, which generates ammonia, and urease activity is required for the establishment of infection. Upregulation of inducible nitric oxide synthase (iNOS) and release of nitric oxide, a proinflammatory molecule, have now been found to be regulated by a component of the urease complex (UreA), suggesting that urease may not only be required for ammonia production but may also mediate inflammation. To facilitate locomotion within gastric mucus and to counteract peristalsis, H. pylori possesses five or six polar flagella, and, similar to urease production, motility is required for persistent infection. FlbA, a component of the H. pylori flagellar secretion apparatus that regulates flagellar biosynthesis, has now been shown to mediate urease activity; thus FlbA may couple urease production and motility, bacterial phenotypes that are necessary for establishment and maintenance of gastric colonization and inflammation.

The vast majority of H. pylori in colonized hosts are free-living, but ~20% bind specifically to gastric epithelial cells, and adherence is required for prolonged inhabitation of the stomach. Analysis of the genome sequences from H. pylori strains 26695 and J99 has revealed that an unusually high proportion (1%) of identified open reading frames is predicted to encode outer membrane proteins (OMPs), which may represent adhesins, and many of these factors also augment disease risk (Table 1). H. pylori BabA, a membrane-bound adhesin encoded by the strain-selective gene babA2, binds the blood-group antigen Lewisb on gastric epithelial cell membranes, and H. pylori baba2 strains increase the risk for gastric cancer precursor lesions and adenocarcinoma. Sialyl-dimeric-Lewisx glycosphingolipid is expressed by neutrophils and gastric epithelial cells and is a marker of gastric dysplasia that is upregulated by chronic gastric inflammation. Sialyl-Lewisx functions as a receptor for H. pylori, and the bacterial adhesin required for binding is SabA, which also has the capacity to activate neutrophils (24). An H. pylori outer membrane protein that may also mediate disease is a 34 kDa proinflammatory protein encoded by oipA. H. pylori strains that contain an in-frame or functional copy of oipA are linked with more severe gastric inflammation, higher bacterial colonization density, enhanced levels of IL-8 in vivo and in vitro, and duodenal ulcer disease. Another adherence-related H. pylori OMP is encoded by hopQ, a gene that exists as either of two alleles, and type I alleles are found significantly more commonly in H. pylori strains isolated from ulcer patients than in strains harvested from patients without ulceration (4). These studies underscore the pivotal role of H. pylori adherence in disease outcome.

The predisposition among H. pylori-infected persons to develop duodenal ulcer disease vs. gastric cancer is dependent, in part, on the topography of gastric inflammation. Antral-predominant gastritis heightens the risk for duodenal ulceration, whereas pangastroitis augments the risk for gastric adenocarcinoma, and there is a distinct dichotomy between these clinical outcomes. Studies focused on adherence have now provided insights into mechanisms through which H. pylori...
Table 1. *H. pylori* OMPs and disease

<table>
<thead>
<tr>
<th>OMP</th>
<th>Host receptor</th>
<th>Disease Association</th>
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<tr>
<td>BabA</td>
<td>Lewis&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Gastric cancer</td>
</tr>
<tr>
<td>SabA</td>
<td>Sialyl-Lewis&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Gastric cancer</td>
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<tr>
<td>HopQ</td>
<td>Unknown</td>
<td>Peptic ulcer disease</td>
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<tr>
<td>OipA</td>
<td>Unknown</td>
<td>Duodenal ulcer disease</td>
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OMP, outer membrane protein.

H. pylori colonizes different regions of the stomach, which likely has an important bearing on disease manifestation. In wild-type mice, *H. pylori* exhibits tropism for gastric mucosa that is devoid of parietal cells (e.g., the boundary between the squamous epithelium and the proximal glandular epithelium), and lymphocytic infiltration is found primarily in this area. In mice having a genetic ablation of parietal cells, however, gastric epithelial progenitor cells synthesize NeuAc2,3Galβ1,4 glycan, a receptor used by *H. pylori*, and this is accompanied by an expansion of bacterial colonization and lymphoid aggregates within the glandular epithelium (23). Thus parietal cell loss that develops within the context of long-term *H. pylori* infection may facilitate gastric injury by delimiting mucosal sites that can sustain colonization.

Histopathological studies in human subjects have consistently demonstrated that *H. pylori* binding and bacterial density are greatest in the upper echelon of gastric glands. This pattern of colonization mirrors the distribution of trefoil factor 1 (TFF1), and *H. pylori* binds avidly to TFF1 dimers (6), suggesting that TFF1 may act as a receptor for this organism in vivo. A specific component of gastric mucin (α1,4-linked N-acetylgalactosamine O-glycan) that is confined to deeper glandular regions rarely colonized by *H. pylori* has now been shown to exert antimicrobial effects against this organism by inhibiting the biosynthesis of cholesteryl-D-glucopyranoside, a major *H. pylori* cell wall component (14). Collectively, these findings indicate that dynamic and specific interactions between *H. pylori* adhesins and host receptors dictate tropism for a particular gastric microniche, which may in turn legislate pathological outcome.

**Evasion of the Host Immune Response by *H. pylori***

If a bacterial species is to persistently colonize a mammalian host, its most formidable challenge is to evade immune clearance. *H. pylori* can survive and replicate within epithelial cells and macrophages, thereby evading host clearance. *H. pylori* resides in epithelial cells within large cytoplasmic vacuoles, and after vacuole evolution, the reappearance of *H. pylori* in the extracellular environment parallels the disappearance of intravacuolar bacteria, suggesting release from intravacuolar sites. At the ultrastructural level, the entry process into epithelial cells occurs via a zipperlike mechanism, as internalized bacteria are bound within phagolysosomes in close association with condensed filamentous actin (15).

Another mechanism through which *H. pylori* may enhance its survival is by limiting the bactericidal effects of proinflammatory molecules, such as nitric oxide. *H. pylori* possesses a gene, rocF, encoding a functional arginase that effectively siphons L-arginine away from the competing host enzyme iNOS. This, in turn, limits the production of iNOS-generated nitric oxide by limiting the availability of L-arginine (12).

Activated neutrophils present within inflamed gastric mucosa generate reactive oxygen and nitrogen species that can induce oxidative DNA damage via formation of DNA adducts, and these reactive species not only damage host tissue, but also have the potential to injure infecting organisms. Therefore, recent studies have focused on the capacity of *H. pylori* to survive within a milieu of oxidative stress. In vitro, *H. pylori* induces expression of a host enzyme, AP endonuclease-1, in gastric epithelial cells, which functions to repair DNA damage (7). *H. pylori* ahpC encodes a protein that catalyzes the reduction of organic peroxides to alcohols, whereas napA encodes an iron-binding neutrophil-activating protein. Inactivation of each of these genes leads to enhanced sensitivity to either paraquat and cumene hydroperoxide or oxygen, respectively, whereas disruption of both genes incurs the highest sensitivity to growth inhibition by organic peroxides and oxygen. Inactivation of ahpC also leads to a severe impairment in the ability of *H. pylori* to infect mice; thus oxidative resistance is critical for successful colonization.

Another level of host defense that may be circumvented by *H. pylori* is innate immunity. Toll-like receptors (TLRs) are an evolutionarily conserved family of eukaryotic receptors that function in innate immunity via recognition of invariant regions in bacterial molecules termed pathogen- or microbes-associated molecular patterns. Eleven different TLRs have been identified in mammals, and although the bacterial ligands for TLRs are distinct, signaling pathways used by these receptors all appear to eventuate in NF-kB activation and proinflammatory gene expression. It is becoming increasingly clear, however, that *H. pylori* has evolved strategies to avoid global activation of this system. For example, TLR4 recognizes bacterial LPS, yet *H. pylori* LPS is relatively anergic compared with that of other enteric bacteria due to Lipid A core modifications. In contrast to flagellins secreted by gram-negative mucosal pathogens such as *Salmonella* or *E. coli* which robustly activate TLR5-mediated proinflammatory responses, *H. pylori* flagellin is not secreted and is non-inflammatory (11). *H. pylori* also hinders another component of the innate immune response, macrophage activation, via production of polyamines, which induces macrophage apoptosis (5).

**Manipulation of the Adaptive Immune Response by *H. pylori* Virulence Constituents**

Despite strategies employed by *H. pylori* to avoid immune clearance, substantial immune activation still occurs as manifested by epithelial signaling, mucosal infiltration by neutrophils, macrophages, and lymphocytes, as well as humoral and cellular recognition of *H. pylori* antigens. Emerging data have now indicated, however, that *H. pylori* can use disease-associated virulence determinants to also downregulate and avoid acquired immune effectors.

The *H. pylori* cag pathogenicity island encodes a type IV secretion system, and bacterial substrates delivered into host cells by this system include 1) peptidoglycan, which is recognized by intracellular Nod1 leading to NF-kB activation (25); and 2) CagA, the product of the terminal gene in the island, which undergoes Src-dependent tyrosine phosphorylation and activates a eukaryotic phosphatase (SHP-2), leading to dephosphorylation of host cell proteins and cellular morphological changes (Fig. 1). Translocation, but not phosphorylation, of...
CagA also leads to disruption of apical-junctional complexes (1). CagA-independent consequences of cag island-mediated epithelial cell contact include production of the proinflammatory cytokine IL-8 and MMP-7, an epithelial-derived matrix metalloproteinase with cancer-initiating properties. As might be predicted by these in vitro observations, H. pylori cag+ strains augment the risk for ulcer disease and gastric cancer compared with cag− strains.

CagY is a surface-exposed component of the H. pylori cag secretion pilus. The NH2 terminus and midregion of CagY contain a striking number of amino acid repeat patterns, suggesting that H. pylori may use repetitive CagY sequences to facilitate immune evasion. In point of fact, Aras et al. (3) demonstrated that cagY undergoes sequence diversification during prolonged in vivo carriage and that H. pylori cagY sequences differ substantially among strains harvested from different individuals. Compared with robust serologic recognition of other H. pylori antigens (e.g., CagA), antibody recognition of surface-exposed CagY is minimal among infected subjects carrying cag+ strains (3), indicating that antigenic variation of CagY allows H. pylori to effectively evade the humoral immune response.

An independent H. pylori locus linked with pathological outcomes is vacA, which encodes a secreted bacterial toxin (VacA). VacA induces multiple structural and functional alterations in epithelial cells in vitro, the most prominent of which is the formation of large intracellular vacuoles. Unlike the cag island, vacA is present in virtually all H. pylori strains examined; however, strains vary in cytotoxic activity, and this variation is primarily due to differences in vacA gene structure. The regions of greatest diversity localize to the 5′-end of vacA (allele types s1a, s1b, s1c, or s2) and the midregion of vacA (allele types m1 or m2). H. pylori s1/m1 strains increase the risk of ulcer disease and gastric cancer compared with vacA s2/m2 strains.

VacA binds to a unique receptor-type protein tyrosine phosphatase (PTPζ), a member of a family of receptorlike enzymes that regulate cellular proliferation, differentiation, and adhesion (9). Oral delivery of VacA induces gastric inflammation, hemorrhage, and ulcers but only in wild-type PTPζ+/+ mice, and in vitro, VacA treatment of PTPζ−/−, but not PTPζ+/−, cells induces cellular detachment, which may contribute to H. pylori-induced ulcerogenesis (9). In addition to vacuolation and cellular detachment, VacA induces gastric epithelial cell apoptosis and functions as a transmembrane pore, permeabilizing host epithelial cells to urea, which may allow H. pylori to manipulate the pH of its environment by generating ammonia. When added to polarized epithelial cell monolayers, VacA increases paracellular permeability to organic molecules, iron, and nickel. These data indicate that VacA can induce multiple physiological consequences that influence pathogenesis.

Intriguing recent observations also indicate that VacA contributes to evasion of the adaptive immune response. VacA can actively suppress proliferation and activation of transformed Jurkat T cells in vitro via inhibition of nuclear factor of activated T cell (NFAT)-mediated IL-2 signaling (10). VacA can also block activation of primary human T cells, effects that are dependent on the VacA NH2-terminus region but independent of NFAT (22). Thus VacA interferes with T cell proliferation and activation via multiple mechanisms, which likely contributes to the longevity of H. pylori colonization.

H. pylori induces inappropriate and ineffective T cell polarization

CD4+ T cells can be broadly divided into two functional subsets, type 1 (Th1) and type 2 (Th2) T helper cells, each of which are defined by distinct patterns of cytokine secretion. Th1 cells produce IL-2 and IFN-γ and promote cell-mediated immune responses, whereas Th2 cells secrete IL-4, IL-5, IL-6, and IL-10 and induce B cell activation and differentiation. In general, most intracellular bacteria induce Th1 responses, whereas extracellular pathogens stimulate Th2-type responses. Although the acquired immune response to H. pylori is composed of both Th1- and Th2-type cells, cytokine profiles indicate a Th1 predominance. This is somewhat counterintui-
tive based on the fact that infection is accompanied by an exuberant humoral response, but studies now suggest that this Th1-biased response is dysfunctional and may play an important role in pathogenesis.

*H. pylori* infection of IFN-γ-deficient mice leads to decreased levels of gastric inflammation and atrophy compared with wild-type mice, and in vivo neutralization of IFN-γ in mice infected with *H. felis* similarly reduces the severity of gastritis. Certain strains of mice (C57BL/6) infected with *H. felis* that mount a polarized Th1-type response develop extensive gastric inflammation, whereas genetically distinct murine strains (BALB/c) that respond to infection with a Th2-like response develop only minimal gastritis. Adoptive transfer of Th1-type cells from *Helicobacter*-infected donor mice into infected recipients increases the severity of gastritis, whereas transfer of Th2-type lymphocytes reduces colonization density. Consistent with these results, antecedent challenge with a helminth (*Heligmosomoides polygyrus*) that induces a Th2-type reaction significantly attenuates the development of Th1-mediated gastritis and atrophy in response to *H. felis* (8), whereas induction of a Th1 response in a Th2-responding host (BALB/c mice) enhances gastric injury following infection with *H. felis* (21).

Dendritic cells may be involved in the aberrant adaptive response to *H. pylori*. C-type lectins are dendritic cell surface receptors that recognize carbohydrate structures and, on binding, internalize pathogens for Ag processing and presentation to T cells. The dendritic cell-specific C-type lectin ICAM-3-grabbing nonintegrin (DC-SIGN, CD209) is involved in binding of the HIV-1 envelope glycoprotein to enhance infection of T cells. Common features of pathogens that interact with DC-SIGN include chronicity and the ability to manipulate the Th1/Th2 response, and binding of mannosylated and fucosylated surface glycans of *H. pylori* to DC-SIGN has now been reported (2). *H. pylori* also induces the release of Th1-type cytokines from dendritic cells in vitro, suggesting that interactions between *H. pylori* and dendritic cells may influence Ag presentation as well as cytokine secretion.

Cyclooxygenases catalyze key steps in the conversion of arachidonic acid to endoperoxide (PGH₂), a substrate for a variety of prostaglandin synthases that, in turn, catalyze the formation of prostaglandins and other eicosanoids. Prostaglandins regulate a diverse array of physiological processes including immunity. Three isoforms of cyclooxygenase (COX) have now been identified, each possessing similar activities, but differing in expression characteristics and inhibition profiles by NSAIDs. COX-1 and COX-3 (a splice variant of COX-1) are expressed constitutively in many cells and tissues. COX-2 expression can be induced by a variety of growth factors and proinflammatory cytokines in a number of pathophysiological conditions. COX-2 expression is increased in epithelial cells cocultured with *H. pylori* and within gastric mucosa of *H. pylori*-infected individuals. COX-2 expression is further increased within *H. pylori*-induced premalignant (atrophic gastritis and intestinal metaplasia) and malignant (adenocarcinoma) lesions, and COX inhibitors such as aspirin and other nonsteroidal anti-inflammatory drugs decrease the risk for distal gastric cancer. Induction of COX-2 by *H. pylori* has now been shown to suppress Th1-mediated responses in vitro (17). With the use of human peripheral blood mononuclear cells, Meyer et al. (17) demonstrated that *H. pylori* induces COX-2 expression and PGE₂ production. In this system, inhibition of COX-2 potentiated a Th1-type response against *H. pylori* and concomitantly reduced Th2-type responses, whereas addition of exogenous PGE₂ had a reciprocal effect. Collectively, these data are consistent with a model in which an ineffective Th1-type response against *H. pylori* facilitates persistence and the development of gastric inflammation and injury.

**H. pylori** strains isolated from different individuals exhibit substantial genetic diversity, which is consistent with a panmictic or freely recombining population structure. Putative mechanisms for the generation of diversity include a high level of spontaneous mutation occurring over a long period within a highly restricted niche as well as frequent intragenic and intergenic recombination. A previous comparison of the genetic content of different *H. pylori* strains using microarray analysis revealed that 22% of genes are strain specific (20), and genetically unique derivatives of a single *H. pylori* strain are present simultaneously within an individual human host and can modify their genetic composition over time (13). An important biological question is whether this extraordinary degree of genetic diversity contributes to longevity of infection.

The *H. pylori* protein RuvC is an endonuclease that functions to resolve recombinant junctions into nicked duplex products, and this molecule is critical for promoting genetic diversity. To determine whether inactivation of *ruvC* altered the ability of *H. pylori* to colonize the stomach, Loughlin et al. (16) infected CD1 mice with the rodent-adapted wild-type *H. pylori* strain SS1 or an isogenic *ruvC*⁻ null mutant derivative that was severely constrained in its ability to undergo homologous recombination. At 7 days postinoculation, colonization density of the *H. pylori* *ruvC*⁻ mutant began to decline and no mutant strains were recovered from any challenged animal by 36 days postchallenge. Of great interest, this same group now demonstrated that wild-type *H. pylori* elicits a Th2-type response in this model, whereas the *H. pylori* *ruvC*⁻ mutant induces a Th1-type response that results in complete clearance of infection (19). These findings provide the first evidence that recombinational processes contributing to *H. pylori* genetic diversity can manipulate the host immune response, which may play a vital role in the establishment, but more importantly, the maintenance of long-term survival within the stomach.

In summary, the host immune response is insufficient to eliminate *H. pylori* from the gastric niche, which correspondingly increases the risk for pathological sequelae such as peptic ulceration and distal gastric cancer. Due to the substantial morbidity and mortality associated with *H. pylori*-induced diseases, research efforts will continue to focus on delineating precise mechanisms through which this pathogen persists within its host and induces gastric inflammation. The excitement for future investigation within this field is palpable because analytical tools now exist, including genome sequences (*H. pylori* and human), measurable phenotypes, and practical animal models, to discern the fundamental biological basis of *H. pylori*-associated diseases. This will not only permit the development of more effective treatments for disease states associated with gastric inflammation, but will also serve as a
paradigm for the role of chronic inflammation in the genesis of other clinical sequelae within the gastrointestinal tract.

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