Helicobacter pylori and Gastric Cancer: Factors That Modulate Disease Risk

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INTRODUCTION

Helicobacter pylori

Less than 3 decades ago, Robin Warren and Barry Marshall definitively identified *Helicobacter pylori* by culturing an organism from gastric biopsy specimens that had been visualized for almost a century by pathologists (196). In 1994, *H. pylori* was recognized as a type I carcinogen, and now it is considered the most common etiologic agent of infection-related cancers, which represent 5.5% of the global cancer burden (239). In 2005, Marshall and Warren were awarded the Nobel Prize of Medicine for their seminal discovery of this bacterium and its role in peptic ulcer disease.

H. pylori is a Gram-negative bacterial pathogen that selectively colonizes the gastric epithelium. The bacterium is urease, catalase, and oxidase positive, is spiral shaped, and possesses 3 to 5 polar flagella that are used for motility. In addition, the majority of *H. pylori* strains express virulence factors that have evolved to affect host cell signaling pathways. Among many unique characteristics of *H. pylori*, one of the most remarkable is its capacity to persist for decades in the harsh gastric environment due to an inability of the host to eliminate the infection. Unlike other viruses and bacteria, *H. pylori* has evolved the ability to colonize the highly acidic environment found within the stomach by metabolizing urea to ammonia via urease, which generates a neutral environment enveloping the bacterium (332). Indeed, evidence now supports the tenet that *H. pylori* has coexisted with humans for tens of thousands of years, with genetic studies indicating that humans have been colonized with *H. pylori* for at least 58,000 years (176).

Approximately half of the world's population is infected with *H. pylori*, and the majority of colonized individuals develop coexisting chronic inflammation. In most persons, *H. pylori* colonization does not cause any symptoms (242). However, long-term carriage of *H. pylori* significantly increases the risk of developing site-specific diseases. Among infected individuals, approximately 10% develop peptic ulcer disease, 1 to 3% develop gastric adenocarcinoma, and <0.1% develop mucosa-associated lymphoid tissue (MALT) lymphoma (244). At early stages, gastric MALT lymphoma can be cured completely by eradication of *H. pylori* and therefore is considered the first clonal lesion which can be eliminated by treatment with antibiotics (294).

The link between *H. pylori* and gastric cancer was a matter of debate for a number of years. However, several studies, including a study of 1,526 Japanese patients, have now provided clear evidence that *H. pylori* infection significantly increases gastric cancer risk (319). Uemura et al. (319) reported that gastric cancer developed in approximately 3% of *H. pylori*-infected patients, compared to none of the uninfected patients. Eradication of *H. pylori* significantly decreases the risk of gastric cancer in infected individuals without premalignant lesions. Randomized prospective studies demonstrated that eradication significantly reduced the presence of premalignant lesions,

providing additional evidence that this organism has an effect on early stages of gastric carcinogenesis (200, 342). In experimentally challenged Mongolian gerbils, eradication of *H. pylori* resulted in a significant attenuation of the progression toward gastric cancer (224, 264). Taken together, these studies support an unequivocal role for *H. pylori* in the development of gastric cancer and indicate that anti-*H. pylori* therapy may be an effective means of gastric cancer prevention.

Though *H. pylori* infection can be found in all regions of the world, rates of colonization vary considerably, with higher rates present in developing countries than in developed areas (87). Most infections are thought to be acquired in childhood via the fecal-oral or oral-oral mode of transmission (85, 87). The variable outcomes of *H. pylori* infection likely depend on factors such as strain-specific bacterial constituents, inflammatory responses governed by host genetic diversity, or environmental influences, which ultimately influence the interactions between pathogen and host (33).

Gastric Cancer

Almost 1 million cases of gastric cancer are diagnosed each year, establishing this disease as the fourth most common cancer worldwide. This is the second leading cause of cancer-related deaths, and approximately 700,000 people succumb each year to gastric adenocarcinoma (239). In some regions of the world, gastric carcinoma is the most common malignancy, and in Japan, the incidence of gastric cancer is almost 10-fold higher than rates observed in the United States. Typically, the diagnosis of gastric cancer is delayed by a lack of early specific symptoms, and most patients are diagnosed after cancer has invaded the muscularis propria. This may be one explanation for why the 5-year survival rate for gastric cancer in the United States is less than 15% (57).

Histologically, two distinct variants of gastric carcinoma have been identified: diffuse-type gastric cancer, which consists of individually infiltrating neoplastic cells that do not form glandular structures; and intestinal-type adenocarcinoma, which progresses through a series of well-defined histological steps and was first described in 1975 (56) (Fig. 1). Intestinal-type adenocarcinoma is initiated by the transition from normal mucosa to chronic superficial gastritis; this is followed by atrophic gastritis and intestinal metaplasia, finally leading to dysplasia and adenocarcinoma (55, 289). This form of gastric cancer affects men twice as commonly as women and occurs in men with a mean age of 50.4 years and in women with a mean age of 47.7 years (59, 128). Corpus-predominant gastritis predisposes individuals toward gastric cancer, which is thought to be due in part to decreased acid secretion. In contrast, infection primarily of the gastric antrum results in increased acid production and predisposes individuals to duodenal ulcer disease, which is associated with a decreased risk of gastric cancer (23).
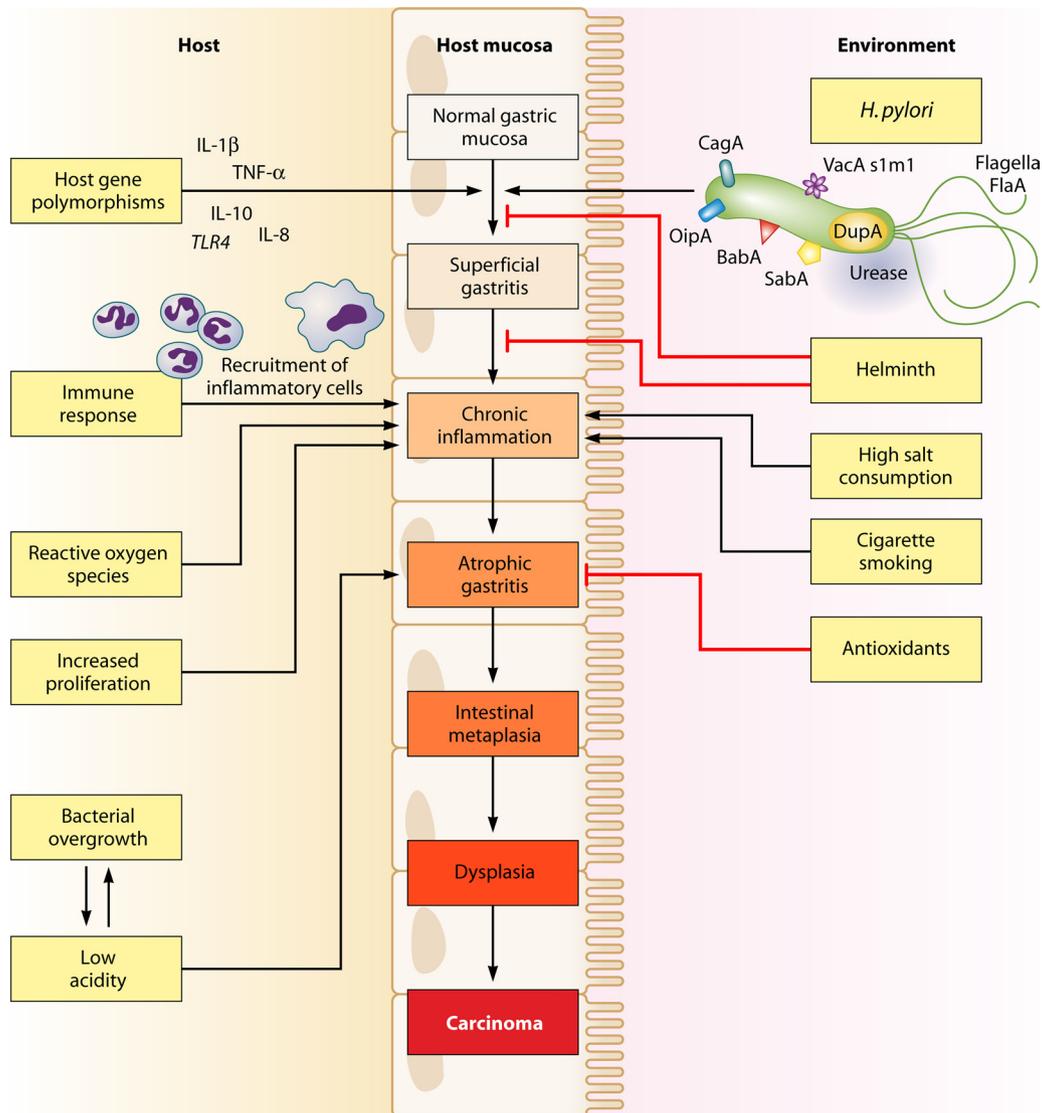
FIG. 1. Multifactorial pathway leading to gastric carcinoma. Many host, bacterial, and environmental factors act in combination to contribute to the precancerous cascade leading to development of gastric cancer.

H. PYLORI VIRULENCE FACTORS

cag PAI

Due to the genetic heterogeneity present within *H. pylori* genomes, bacterial virulence factors likely play an important role in determining the outcome of *H. pylori* infection. The *cag* pathogenicity island (*cag* PAI) is a 40-kb DNA insertion element which contains 27 to 31 genes flanked by 31-bp direct repeats and encodes one of the most intensely investigated *H. pylori* proteins, CagA (7, 43, 60). CagA was initially identified in the early 1990s, and expression of CagA was found to be associated strongly with peptic ulceration (62, 67). Due to its association with clinical disease, the *cag* PAI is now a well-characterized *H. pylori* virulence determinant, and CagA is frequently used as an indicator of the presence of the entire *cag* PAI.

Approximately 60 to 70% of Western *H. pylori* strains and almost 100% of East Asian strains express CagA (10, 43, 310).

Although all *H. pylori* strains induce gastritis, strains that contain the *cag* PAI (*cag*⁺) augment the risks for severe gastritis, atrophic gastritis, and distal gastric cancer compared to those with strains that lack the *cag* island (*cag*-deficient mutants) (34, 62, 67, 68, 167, 240, 245, 252, 265, 287, 311, 325). At least 18 *cag* genes encode components of a bacterial type IV secretion apparatus which functions to export bacterial proteins across the bacterial membrane and into host gastric epithelial cells.

CagA

H. pylori strains are frequently segregated into *cagA*-positive and *cagA*-negative strains, depending on the presence or absence of the terminal gene product of the *cag* island, CagA. The *H. pylori* CagA protein is a 120- to 140-kDa protein that is translocated into host cells by the type IV *cag* secretion system after bacterial attachment. Once inside the host cell, CagA is tyrosine phosphorylated at glutamate-proline-isoleucine-

tyrosine-alanine (EPIYA) motifs and induces cell morphological changes, initially termed "the hummingbird phenotype," which are associated with increased cellular migration (20, 226, 279, 292, 293) (see "CagA phosphorylation-dependent host cell signaling").

To date, four distinct EPIYA motifs (EPIYA-A, -B, -C, and -D) have been identified within the carboxy-terminal polymorphic region of CagA, and they are distinguished by different amino acid sequences surrounding the EPIYA motif (128, 134, 215). EPIYA-A and -B motifs are present in strains throughout the world, whereas EPIYA-C is typically found only in strains from Western countries (Europe, North America, and Australia). The number of EPIYA-C sites can vary; however, most CagA proteins contain a single EPIYA-C site (A-B-C type), and EPIYA-A and EPIYA-B sites are phosphorylated to a lesser extent than EPIYA-C sites. In Western strains, an increased number of CagA EPIYA-C sites is an important indicator of the risk of developing gastric cancer (31). The EPIYA-D motif is found almost exclusively in East Asian strains (from Japan, South Korea, and China), and strains containing this motif induce larger amounts of interleukin-8 (IL-8) from gastric epithelial cells than do strains harboring Western A-B-C-type CagA (19, 128).

CagA phosphorylation-dependent host cell signaling. Once it is phosphorylated by members of the Abl and Src families of kinases, phospho-CagA targets and interacts with numerous intracellular effectors. Phospho-CagA activates a eukaryotic tyrosine phosphatase (SHP-2), leading to sustained activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), Crk adaptor (133), and C-terminal Src kinase, in a tyrosine phosphorylation-dependent manner in which East Asian A-B-D-type CagA exhibits stronger binding activity for SHP-2 than Western A-B-C-type CagA (133). Interactions of phospho-CagA with C-terminal Src kinase rapidly activate a negative feedback loop to downregulate Src signaling (315).

In a human gastric adenocarcinoma cell line (AGS), translocation and subsequent phosphorylation of CagA result in the "hummingbird phenotype," a phenotype associated with cell elongation and cell scattering (209, 279). In AGS cells, the interaction between phospho-CagA and SHP-2 increases the duration of ERK activation, in a manner independent of Ras or phosphatidylinositol 3-kinase (PI3K), and results in cell elongation (132). The interaction between CagA and SHP-2 also dephosphorylates and inactivates focal adhesion kinase (FAK), resulting in cell elongation (316). Phosphorylated CagA also induces cell elongation by inducing a defect in cell retraction; however, the signaling molecules required for this phenotype remain undefined (38). In addition, the catalytic activity of c-Src is inhibited by phosphorylated CagA, which leads to tyrosine dephosphorylation of the actin binding proteins cortactin, ezrin, and vinculin, leading to cell elongation (208, 280, 281).

CagA phosphorylation-independent host cell signaling. Nonphosphorylated CagA also exerts effects within the cell that contribute to pathogenesis. Translocation, but not phosphorylation, of CagA leads to aberrant activation of β-catenin, disruption of apical-junctional complexes, and a loss of cellular polarity (13, 27, 101, 212, 269, 303). Nonphosphorylated CagA targets the cell adhesion protein E-cadherin, the hepatocyte growth factor receptor c-Met, phospholipase C gamma (PLC-

γ), the adaptor protein Grb2, and the kinase partitioning-defective 1b/microtubule affinity-regulating kinase 2 (PAR1b/MARK2) (53, 205, 212, 269), which leads to proinflammatory and mitogenic responses, disruption of cell-cell junctions, and loss of cell polarity. Nonphosphorylated CagA associates with the epithelial tight junction scaffolding protein zonula occludens 1 (ZO-1) and the transmembrane protein junctional adhesion molecule A (JAM-A), leading to nascent but incomplete assembly of tight junctions at ectopic sites of bacterial attachment (13). Recently, CagA was shown to directly bind PAR1b/MARK2, a central regulator of cell polarity, and to inhibit its kinase activity as well as to dysregulate mitotic spindle formation, thus promoting a loss of cell polarity (see "Apical-Junctional Complexes") (179, 269, 320). While it is evident that non-tyrosine-phosphorylated mutant forms of CagA exert effects within gastric epithelial cells, to our knowledge, there is currently no direct evidence for nonphosphorylated CagA within the host cell.

CagA is one of the most intensely investigated *H. pylori* proteins, and to date, it is the only bacterial effector protein known to be translocated by the type IV *cag* secretion system. As discussed above, studies with animal and cell culture models have provided evidence for the importance of CagA in *H. pylori* pathogenesis and have highlighted the vast array of host cell functions with which CagA may interfere. The generation of transgenic mice expressing CagA has now provided more direct evidence for a causal relationship between CagA and oncogenesis by demonstrating that transgenic expression of CagA leads to gastric epithelial cell proliferation and carcinoma. These changes were not observed in mice expressing phosphorylation-resistant CagA (231). Overall, there is strong evidence that CagA functions as a bacterial oncoprotein in mammals. However, experimental models that are currently available have not provided all of the answers. Cell culture and animal models often provide conflicting data, and pathological changes reported for transgenic CagA mice occurred in the absence of inflammation, which is in stark contrast to what is seen in humans (231). In addition, only a small fraction of individuals colonized by CagA-positive *H. pylori* develop gastric cancer. Much remains to be learned about the circumstances that coalesce to permit CagA to initiate carcinogenesis. Until very recently, it was unclear how CagA is actually delivered into the host cell. However, Murata-Kamiya and colleagues have now reported that *H. pylori* induces the appearance of a host phospholipid, phosphatidylserine, on the external leaflet of the plasma membrane, where CagA can specifically interact and gain entry into the cells (211). Fertile areas of future research will include studies to determine the specific mechanism by which CagA is internalized and when during chronic infection CagA is translocated into host cells.

Peptidoglycan

In addition to CagA, the *cag* secretion system can also deliver components of *H. pylori* peptidoglycan into host cells. Peptidoglycan interacts with the host intracellular pattern recognition molecule Nod1, which acts as a sensor for peptidoglycan components originating from Gram-negative bacteria. The interaction of *H. pylori* peptidoglycan with Nod1 leads to activation of NF-κB-dependent proinflammatory responses, such

as secretion of IL-8 (323) or β-defensin-2 (37). *H. pylori* translocated peptidoglycan has been shown to activate other host signaling pathways that are associated with an increased risk for developing gastric cancer. For example, a recent study demonstrated that *H. pylori* translocated peptidoglycan can activate PI3K-AKT signaling, leading to decreased apoptosis and increased cell migration (214). Another study revealed that intracellular sensing of *H. pylori* peptidoglycan components triggers an intracellular signaling cascade, which culminates in the production of type I interferon (IFN) (331).

VacA Toxin

An independent *H. pylori* locus linked with increased disease risk is *vacA*, which encodes the secreted toxin VacA (61, 64, 247, 276). VacA was first identified as a proteinaceous cytotoxin that induced intracellular vacuolation of cultured cells (173). It was later purified to homogeneity from *H. pylori* broth culture supernatants and was identified as a protein of approximately 87 kDa in its denatured form (61). VacA suppresses T-cell responses to *H. pylori*, which may contribute to the longevity of infection (35, 111, 299).

The *vacA* gene is present in the majority of *H. pylori* strains; however, considerable differences in vacuolating activities are observed between strains. This variation is attributed to variations in *vacA* gene structures within the signal (s) region, the middle (m) region, and the more recently identified intermediate (i) region, which is located between the s and m regions (259). The s region is stratified into s1 and s2 subtypes and encodes a component of the signal peptide and the N terminus of the mature protein. The m region partially encodes the 55-kDa C-terminal subunit and is classified as either the m1 or m2 type. *vacA* s1/m1 chimeric strains induce greater vacuolation than do s1/m2 strains, and there is typically no vacuolating activity in s2/m2 strains (24, 61, 259, 321). In Western populations, the *vacA* s1/m1 allele is strongly associated with duodenal and gastric ulcer disease and with gastric cancer (24, 25, 203). East Asian strains are almost all *vacA* s1/m1 and, as predicted, are not associated with any specific clinical outcome. There are two i region subtypes, i1 and i2; the i region plays a functional role in vacuolating activity, since *vacA* s1/i1/m2 strains are vacuolating types and *vacA* s1/i2/m2 strains do not induce vacuolation. All s1/m1 *vacA* alleles are of type i1, all s2/m2 alleles are of type i2, and s1/m2 alleles can be either i1 or i2 (259). In a study of 73 *H. pylori*-infected Iranian patients, colonization with *vacA* i1 strains was strongly associated with gastric cancer. This association with gastric cancer may be stronger than associations of *vacA* s or m types or *cag* status (259). Among *H. pylori* strains isolated from patients in Iraq and Italy, *vacA* i1 strains were associated with gastric ulcer disease (31, 74, 141). However, in East Asian and Southeast Asian populations, where the incidence of gastric cancer is high, *vacA* i-region subtype is not associated with risk of disease (228).

Recently, a deletion of 81 bp between the m region and the i region was identified and termed the d region; d1 strains have no deletion, while strains of the d2 type contain a 69- to 81-bp deletion. For a small number of Western strains, but not East Asian strains, *vacA* d1 type was significantly associated with neutrophil infiltration and gastric mucosal atrophy, potentially making the d region genotype another risk locus for gastric cancer and peptic ulceration in Western strains (229).

Consequences of VacA within the host cell. Similar to elements encoded by the *cag* PAI, VacA exerts multiple effects on epithelial cell structure and results in phenotypes that include disruption of gastric epithelial barrier function and modulation of the inflammatory response. Other effects of VacA include disruption of late endosomal compartments, which results in vacuole formation *in vitro* (173, 237), and targeting of mitochondria, leading to a decrease in mitochondrial transmembrane potential, the release of cytochrome *c*, activation of caspase-8 and caspase-9, and induction of apoptosis *in vitro* (63, 107, 190, 243, 336).

One of several receptors that VacA binds to on gastric epithelial cells is the receptor-type protein tyrosine phosphatase RPTPβ. This receptor regulates cell proliferation, differentiation, and adhesion, all of which likely play roles in ulcerogenesis (105). Oral administration of acid-activated and then neutralized VacA to wild-type RPTPβ^{+/+} mice has a profound effect on the gastric epithelium. Within 2 days of VacA administration, heavy bleeding develops in the stomach, which leads to development of gastric ulcers and gastric atrophy. In sharp contrast, RPTPβ^{−/−} mice receiving VacA are resistant to the development of gastric damage (105). Interestingly, in primary cultures of cells isolated from either RPTPβ^{−/−} or RPTPβ^{+/+} mice, VacA induces vacuolation. However, only cells isolated from RPTPβ^{+/+} mice detach from Matrigel in response to VacA, suggesting that VacA induces gastric ulcers through RPTPβ signaling, not vacuolation (99).

Recent reports suggest that CagA is able to downregulate the effects of VacA on host cell vacuolation, and conversely, VacA may downregulate CagA activity (232, 308). Mechanistically, Oldani et al. determined that tyrosine-phosphorylated CagA blocks VacA trafficking, preventing it from reaching its intracellular target and inducing vacuole formation. Via a separate mechanism, unphosphorylated CagA antagonized vacuolation by blocking VacA activity at the mitochondria (232). Conversely, VacA antagonizes the effects of CagA on cell scattering and elongation by inactivating epidermal growth factor receptor (EGFR) and HER2/Neu, which suppresses activation of ERK1/2 mitogen-activated protein (MAP) kinase and the hummingbird phenotype (308). These findings further highlight mechanisms through which *H. pylori* can avoid the induction of excess cellular damage and maintain long-term colonization of the gastric niche.

Adhesins and OMPs

Adherence of *H. pylori* to the gastric epithelium facilitates initial colonization, persistence of infection, and delivery of virulence factors to host epithelial cells. Sequence analysis of six completely sequenced *H. pylori* strains reveals that approximately 4% of the *H. pylori* genome is predicted to encode outer membrane proteins (OMPs), which is significantly more than that for other known bacterial species. OMP expression has been associated with gastroduodenal diseases (as discussed below) and therefore may heighten the risk for developing gastric cancer (73).

BabA. Blood group antigen binding adhesin (BabA) is encoded by the *babA2* gene, which binds to fucosylated Lewis[b]

antigen (Le[b]) on the surfaces of gastric epithelial cells and is the most well-described *H. pylori* OMP (36, 113, 143). Transgenic mice that express Le[b] on pit and surface mucous cells have been used to demonstrate that *H. pylori* attaches to the surfaces of Le[b]-expressing cells (123). The ensuing gastritis is more severe than that seen in nontransgenic mice, despite a comparable colonization density, suggesting that Le[b]-mediated colonization may increase the pathogenic potential of *H. pylori* (123). A recent study utilizing the same system demonstrated that selective pressure is exerted by transgenic Le[b] expression and dictates the pattern of Lewis antigen expression on *H. pylori* lipopolysaccharide (LPS) from Le[x] and Le[y] toward Le[b] (248).

Analyses of binding specificities of *H. pylori* strains from across the world suggest that the BabA adhesin has evolved in response to host mucosal glycosylation patterns to permit *H. pylori* to adapt to its host and to maintain persistent colonization (22, 248). The presence of *babA2* is associated with duodenal ulcer disease and gastric cancer, and when found in conjunction with *cagA* and *vacA* s1 alleles, it is associated with an even greater risk of developing more severe disease (113). More recent analyses of *babA2* as a virulence marker have produced conflicting data on the usefulness of *babA2* expression in predicting clinical outcome, which is most likely dependent on the geographic origin of the *H. pylori* strains. In Portuguese and Thai populations, *babA2* is not a biomarker for peptic ulcer disease or gastric cancer (52, 110). However, for strains isolated from Germany, Turkey, or northern Portugal, *babA2* expression is associated with the severity of gastric disease (26, 86, 113).

SabA and OipA. Sialic acid-binding adhesin (SabA) is an *H. pylori* adhesin that binds to the carbohydrate structure sialyl-Lewis[x] antigen expressed on gastric epithelium and is associated with an increased gastric cancer risk but a reduced risk for duodenal ulceration (354). Sialyl-Lewis[x] expression is induced during chronic gastric inflammation, suggesting that *H. pylori* modulates host cell glycosylation patterns to enhance attachment and colonization (187). *In vitro*, *H. pylori* induces expression of sialyl-Lewis[x] antigens via induction of the gene encoding beta3 GlcNAc T5, a transferase essential for the biosynthesis of Lewis antigens (195). Furthermore, SabA is regulated by phase variation, such that SabA expression can rapidly be switched "on" or "off" to adapt to changes exerted by the gastric niche (354).

Outer inflammatory protein (OipA) is an inflammation-related outer membrane protein (353). *H. pylori* contains either a functional or nonfunctional *oipA* gene, and the presence of a functional gene is significantly associated with the presence of duodenal ulcers, gastric cancer, and increased neutrophil infiltration (102, 354). OipA expression is linked to increased IL-8 production *in vitro* (352). Recent work using Mongolian gerbils infected with wild-type *H. pylori* and an isogenic *oipA* mutant strain demonstrated a role for OipA in induction of the mucosal cytokines IL-1, IL-17, and tumor necrosis factor alpha (TNF-α) and in gastric mucosal inflammation (296). OipA is also involved in upregulation of matrix metalloproteinase 1 (MMP-1), an MMP associated with gastric cancer (347); in inhibition of glycogen synthase kinase 3β (GSK-3β) (304); and in β-catenin translocation to the nucleus (102). Accumulation of β-catenin in the nucleus results in the formation of het-

erodimers with LEF/TCF transcription factors and in transcriptional activation of genes that can influence carcinogenesis.

DupA. Duodenal ulcer promoting gene (*dupA*) is located within the plasticity zone of the *H. pylori* genome and may be another novel virulence marker. Initial analysis of 500 *H. pylori* strains from Colombia, South Korea, and Japan showed an increased risk for duodenal ulcer and a decreased risk for gastric cancer in persons carrying *dupA*-positive strains (178). *In vitro*, DupA increases IL-8 production (178). However, a subsequent study focused on strains from Belgium, South Africa, China, and the United States found no significant relationships between *dupA* expression and duodenal ulcer but a significant association with gastric cancer (18). Comparison of strains from Iran and Iraq indicates that *dupA* expression is significantly associated with duodenal ulceration in strains isolated from Iraq but not in Iranian isolates (141). No association was found between *dupA* expression and gastric cancer or duodenal ulcer in strains from Japan (220) or Sweden (275), but correlations were observed between *dupA* and duodenal ulcer disease or gastric cancer in Chinese strains (275). Collectively, it seems likely that *dupA* may promote duodenal ulceration and/or gastric cancer in some, but not all, populations.

FlaA. *H. pylori* possesses a unipolar bundle of 3 to 5 flagella, which are composed of three structural elements: the basal body, the hook, and the filament (112, 234). The filament acts as a propeller when rotated at its base and is a copolymer of the flagellin subunits FlaA and FlaB (175, 295). FlaA is the predominant subunit, and FlaB is the minor subunit. Mutation of *flaA* results in flagellar truncation and decreased motility *in vitro* (149). *In vivo*, FlaA and other proteins necessary for flagellar assembly are essential for persistent infection in rodent and gnotobiotic piglet models (79, 157, 160). Unlike flagellin from *Salmonella* or other Gram-negative pathogens that colonize mucosal surfaces, *H. pylori* FlaA has low intrinsic activity to activate Toll-like receptor 5 (TLR5), which may contribute to evasion of the host immune response and to persistent colonization (15, 114, 170). Isogenic mutants of *motB*, which encodes the MotB flagellar motor protein and is required for motility, also significantly affect colonization (235). In addition, comparing a noncarcinogenic strain of *H. pylori* to an *in vivo*-adapted carcinogenic strain, the noncarcinogenic strain was found to contain a single nucleotide mutation in FlaA rendering it less motile than the carcinogenic strain (100). Thus, motility appears to be essential for successful gastric colonization and may contribute to pathogenesis.

HOST FACTORS

Host Polymorphisms That Influence the Propensity toward Gastric Cancer Development

IL-1β. *H. pylori* strain-specific constituents are not absolute determinants of virulence, as most persons colonized with disease-associated strains remain asymptomatic. This has underscored the need to define host factors that may also influence pathological outcomes. Critical host responses that influence the progression to *H. pylori*-induced carcinogenesis include gastric inflammation and a reduction in acid secretion (81). A

host effector molecule that interacts with both of these parameters is the Th1 cytokine IL-1β, a pleiotropic proinflammatory molecule that is increased within the gastric mucosa of *H. pylori*-infected persons (222). The IL-1β gene cluster, consisting of *IL-1β* and *IL-1RN* (encoding the naturally occurring IL-1β receptor antagonist), contains a number of functionally relevant polymorphisms that are associated with either increased or decreased IL-1β production, which has permitted case-control studies to be performed that relate host genotypes to disease. El-Omar et al. were the first to demonstrate that *H. pylori*-colonized persons with high-expression IL-1β polymorphisms have a significantly increased risk for hypochlorhydria, gastric atrophy, and distal gastric adenocarcinoma compared to that for persons with genotypes that limit IL-1β expression (82). Importantly, these relationships were present only among *H. pylori*-colonized persons, not uninfected individuals, emphasizing the critical role of host-environment interactions and inflammation in the progression to gastric cancer. Since this initial report, similar findings have been replicated in geographically distinct regions of the world that comprise Caucasian, Asian, and Hispanic populations (106, 185).

Investigations utilizing animal models have confirmed these observational studies of humans. In *H. pylori*-infected Mongolian gerbils, gastric mucosal IL-1β levels increased at 6 to 12 weeks postchallenge, and this was accompanied by a reciprocal decrease in gastric acid output (305). Administration of recombinant IL-1 receptor antagonist normalized acid outputs (305), implicating IL-1β as a pivotal modulator of acid secretion within inflamed mucosa. Gastric tissue levels of IL-1β are similarly higher in colonized human patients possessing high- versus low-expression *IL-1β* polymorphisms, and increased IL-1β levels are significantly related to the intensity of gastric inflammation and atrophy (142). IL-1β transgenic mice overexpressing human IL-1β in parietal cells have been found to develop spontaneous gastritis and dysplasia after 1 year of age and to exhibit increased dysplasia and carcinoma when infected with *Helicobacter felis* (317). Importantly, these findings were linked to activation of myeloid suppressor cells (MDSCs) (317). MDSCs are Gr-1$^+$ CD11b$^+$ immature myeloid cells that have been associated with tumor development and growth (40, 356, 357) and with IL-1β (40, 290). Since IL-1β is a potent inhibitor of acid secretion, is profoundly proinflammatory, is upregulated by *H. pylori*, and is regulated by promoters with informative polymorphisms, this molecule likely plays a critical role in the development of gastric cancer.

TNF-α. In addition to IL-1β, TNF-α is a proinflammatory acid-suppressive cytokine that is increased within *H. pylori*-colonized human gastric mucosa (66). Polymorphisms that increase TNF-α expression have now been associated with an increased risk of gastric cancer and its precursors (83). Oguma et al. recently identified a link between expression of this proinflammatory cytokine and aberrant β-catenin signaling by using transgenic mice that overexpress the β-catenin agonist Wnt1 and develop gastric dysplasia (229a). Within dysplastic mucosa, infiltrating macrophages were observed to be in close apposition to gastric epithelial cells harboring nuclear β-catenin. *In vitro* studies revealed that supernatants from activated macrophages promoted β-catenin signaling in gastric epithelial cells, which was attenuated by inhibition of binding of TNF-α to its cognate receptor on gastric epithelial cells, providing a

potential mechanism through which enhanced levels of TNF-α may augment the risk for gastric cancer.

IL-10. Polymorphisms that reduce the production of anti-inflammatory cytokines may similarly increase the risk for gastric cancer, and low-expression polymorphisms within the locus controlling expression of the anti-inflammatory cytokine IL-10 are associated with an enhanced risk of distal gastric cancer (83). The combinatorial effect of IL-1β, TNF-α, and IL-10 polymorphisms on the development of cancer has also been determined, and risk increases progressively with an increasing number of proinflammatory polymorphisms, to the point that three high-risk polymorphisms increase the risk of cancer 27-fold over baseline (83).

IL-8. Genetic polymorphisms that affect innate immune response genes have also been linked to an increased risk of gastric cancer. High-expression alleles within the promoter region of the chemokine IL-8 gene increase the risk for severe inflammation and premalignant lesions in Caucasian and Asian populations, but this has not been confirmed in all studies. A functional polymorphism within *TLR4* has also been demonstrated to increase the risk for gastric atrophy and gastric cancer in white populations, which may be related to a deficiency in production of the anti-inflammatory cytokine IL-10.

An important question raised by these studies is whether *H. pylori* strain characteristics augment cancer risk exerted by host genotypes. Figueiredo et al. stratified *H. pylori*-infected subjects on the basis of high-expression IL-1β polymorphisms and virulence genotypes of their infecting *H. pylori* strains (94). Odds ratios for distal gastric cancer were greatest for those persons with high-risk host and bacterial genotypes, and specifically, for persons with high-expression IL-1β alleles that were colonized by *H. pylori vacA* s1-type strains, the relative risk for gastric cancer was 87-fold over baseline (94), indicating that interactions between specific host and microbial characteristics are biologically significant for the development of gastric cancer.

On the basis of these case-control studies, it is apparent that *H. pylori* organisms are able to send and receive signals from their hosts, allowing host and bacteria to become linked in a dynamic equilibrium (161, 242). The equilibrium is likely different for each colonized individual, as determined by both host and bacterial characteristics, and may explain why certain *H. pylori* strains augment the risk for carcinogenesis. For example, *cag*$^+$ strains induce severe gastritis, leading to increased production of proinflammatory cytokines, such as IL-1β and TNF-α, that not only can amplify the mucosal inflammatory response but also may inhibit acid production, especially in hosts with polymorphisms that permit high expression levels of these molecules. This creates an environment conducive to the growth of other bacteria that can sustain inflammation and continually induce oxidative stress, thus heightening the risk for gastric cancer.

COX-2

In addition to stimulating cytokine production, *H. pylori* also activates proinflammatory cyclooxygenase (COX) enzymes. Cyclooxygenases catalyze key steps in the conversion of arachidonic acid to endoperoxide (PGH$_2$), a substrate for a vari-
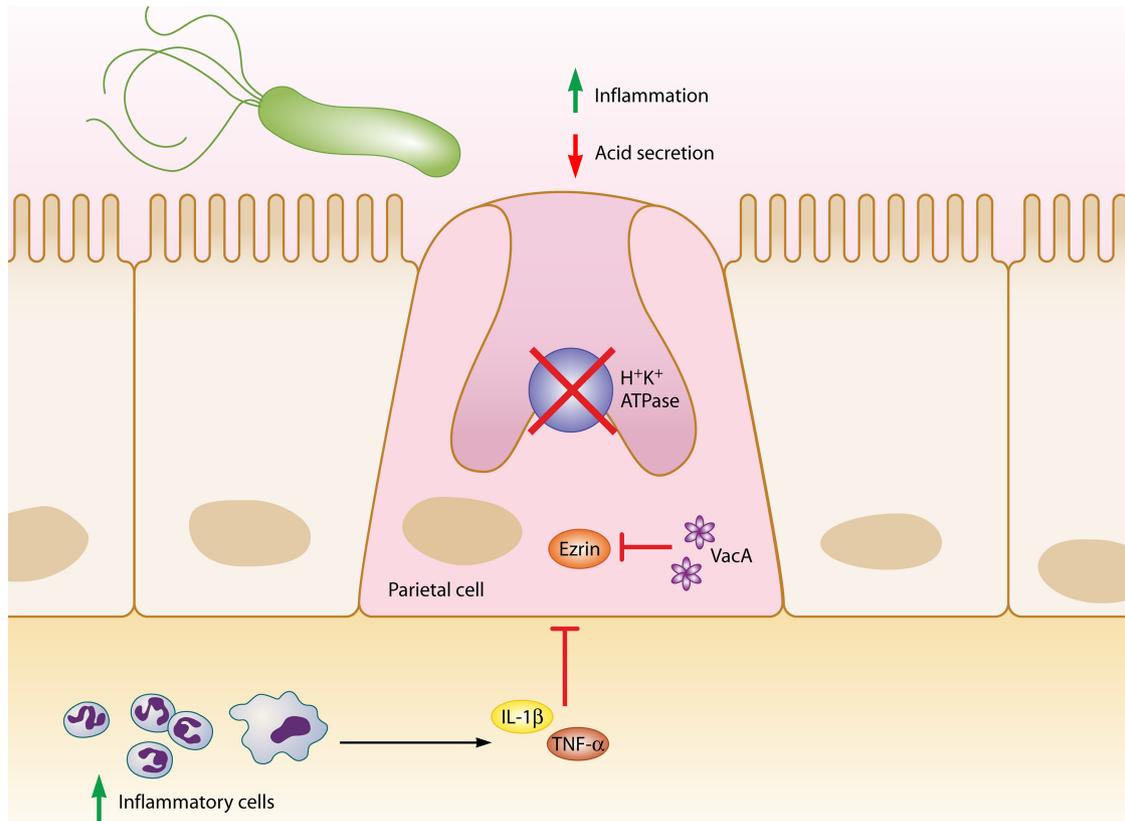
FIG. 2. Relationships between *H. pylori*, inflammation, and acid secretion. *H. pylori* infection can reduce acid secretion and increase inflammation via multiple intermediates. Increased production of IL-1β and TNF-α from inflammatory cells inhibits acid secretion from parietal cells. Acid secretion is also inhibited by repression of H⁺K⁺ ATPase α-subunit promoter activity, in addition to VacA-induced proteolysis of ezrin.

ety of prostaglandin synthases that catalyze the formation of prostaglandins and other eicosanoids (122). Prostaglandins regulate a diverse array of physiologic processes, including immunity and development (122), and different isoforms of cyclooxygenase have been identified, each possessing similar activities but differing in expression characteristics and inhibition profiles for nonsteroidal anti-inflammatory drugs (NSAIDs). COX-1 is expressed constitutively in many cells and tissues (207, 337), while COX-2 expression is inducible and can be stimulated by a variety of growth factors and proinflammatory cytokines, such as TNF-α, IFN-γ, and IL-1 (337). COX-2 expression is increased in gastric epithelial cells cocultured with *H. pylori* (150, 263) and within infected human gastric mucosa (103, 274). COX-2 expression is further increased within gastric premalignant and malignant lesions (260, 300), and COX inhibitors such as aspirin and other NSAIDs decrease the risk of distal gastric cancer (8, 92). *H. pylori* also activates phospholipase A_2, an enzyme that catalyzes the formation of the prostaglandin precursor arachidonic acid, both *in vitro* and *in vivo* (217, 249). The capacity of COX-2-generated products to promote neoplasia is well described, and specific mechanisms utilized by these molecules include stimulation of proliferation with inhibition of apoptosis (which leads to a heightened retention of mutagenized cells), promotion of cellular adhesion, stimulation of angiogenesis, and cellular transformation (229, 231).

Acid Secretion

Gastrin, acetylcholine, and histamine are major stimulants of gastric acid secretion. In the gastric corpus, gastrin acts directly on parietal cells and indirectly via histamine release from ECL cells, which in turn activates histamine-H_2 receptors on the parietal cell to elicit the release of acid. Acetylcholine acts directly on M3 receptors on the parietal cell and indirectly through histamine release from the ECL cell and inhibition of somatostatin release from D cells (277). Parietal cell stimulation elicits an extensive conformational transformation whereby the tubulovesicles of the resting parietal cell are transformed into secretory canaliculi. The H⁺K⁺ ATPase is the primary gastric proton pump, and in unstimulated parietal cells, it is located in tubulovesicles in the cytoplasm. Upon stimulation, the H⁺K⁺ ATPase is translocated to the apical membrane to mediate secretion of acid (96).

H. pylori can inhibit or stimulate acid secretion, depending on the context of infection. Acute infection is usually associated with hypochlorhydria as a result of increased production of the proinflammatory cytokine IL-1β and inhibition of H⁺K⁺ ATPase α-subunit promoter activity (277) (Fig. 2). Indeed, recent work suggests that *H. felis* infection leads to a decrease in acid production via increased IL-1β acting at the parietal cell IL-1β receptor, which subsequently acts to de-

crease sonic hedgehog gene expression and to inhibit acid secretion (326). VacA also induces hypochlorhydria by proteolysis of ezrin, which disrupts apical membrane-cytoskeleton interactions in gastric parietal cells that are required to translocate the H^+K^+ ATPase for acid secretion (329). *H. pylori* may also decrease acid secretion through repression of H^+K^+ ATPase α-subunit gene expression by ERK1/2-mediated activation and translocation of NF-κB to the nucleus (271).

Chronic *H. pylori* infection may result in hypochlorhydria or hyperchlorhydria, depending on the severity and distribution of gastritis. Most patients infected long-term develop pangastritis associated with hypochlorydria, which may progress to gastric ulceration and/or adenocarcinoma. Conversely, antral predominant gastritis occurs in approximately 12% of chronically infected patients and is characterized by hyperchlorhydria, which may lead to duodenal ulcer disease (23).

Oxidative Damage

A potential contributing factor in the inflammation-to-carcinoma sequence is the generation of oxidative stress. Oxidative DNA damage induced by *H. pylori* infection has been well documented for gastritis tissues (28, 90). While this may derive from infiltrating neutrophils, DNA damage has been demonstrated in gastric epithelial cell lines directly exposed to *H. pylori* (225). The generation of reactive oxygen species in the gastric epithelium may also contribute to dysfunction of these cells, and the oxidative stress response in gastric epithelial cells has been linked to the presence of the *cag* PAI (72).

As discussed below, polyamines have been implicated in the pathogenesis of *H. pylori* infection. One specific aspect of this is that oxidation of the polyamine spermine by the enzyme spermine oxidase (SMO; originally termed polyamine oxidase 1) is induced by upregulation of SMO in gastric epithelial cells, and this results in generation of H_2O_2 (350). Various metabolites of H_2O_2, such as hydroxyl radicals (OH $\cdot$), can be highly damaging to macromolecules within cells, including DNA. Inhibition or small interfering RNA (siRNA) knockdown of SMO blocks both apoptosis and DNA damage in gastric epithelial cells (350). In addition, *H. pylori*-activated macrophages also exhibit marked upregulation of SMO, which causes both apoptosis due to mitochondrial membrane depolarization and release of H_2O_2 into the extracellular space (48), which can contribute to oxidative stress in adjacent epithelial cells.

Role of the host immune response in *H. pylori*-induced carcinogenesis. In considering the importance of host immune/inflammatory responses in the pathogenesis of *H. pylori*-induced gastric cancer, it is essential to evaluate the potential mechanisms for how immune dysregulation contributes to neoplastic transformation. In many diseases, including those resulting from chronic infections, dysregulation of the immune system is a central component. A signature feature of *H. pylori* infection is the presence of chronic active gastritis, characterized by both chronic (lymphocytic) and active (neutrophilic) forms of inflammation (119, 196). In the majority of cases, the bacterium remains in the stomach for the life of the host, indicating that the immune response is ineffective. Furthermore, the presence of inflammation for decades supports the notion that the immune response is indeed dysregulated.

General Considerations for Innate and Adaptive Immunity

Innate immunity. Innate immunity refers to responses that do not require previous exposure to the immune stimulus and represents the first line of defense in the response to pathogens. A major advance in this field has been the elucidation of the TLRs, which are activated by recognition of pathogen-associated molecular patterns (PAMPs) (6). Nonspecific activation by stimuli from microorganisms can lead to important antimicrobial effects but can also result in inflammation and injury due to release of inflammatory mediators such as cytokines, reactive oxygen species, and nitric oxide (NO).

Adaptive immunity. The adaptive immune response is considered a predetermined response to a previously identified immunologic stimulus. Thus, the response is specific to a particular pathogen and involves immunologic memory. However, the lines between adaptive and innate immunity are blurred by the close interactions between pathways, such that stimulation of antigen-presenting macrophages and dendritic cells (DCs) leads to activation and recruitment of lymphocytes and the development of T-helper (Th)-cell-specific responses.

Differentiation of Th cells (1) involves clonal expansion caused by engagement of the T-cell receptor (213). Th cells are believed to differentiate into two major $CD4^+$ functional classes, namely, Th1 cells, which produce a set of cytokines that include IFN-γ and IL-2, and Th2 cells, which produce cytokines such as IL-4, IL-5, IL-10, and IL-13 (91). Th1 cells generate cell-mediated immunity, which is important in protection against intracellular parasites, while Th2 responses are associated with humoral immunity and protection against intestinal helminths (213). In addition to the Th1/Th2 paradigm, a third class of $CD4^+$ cells has been discovered (126) and is linked to the etiopathogenesis of inflammatory bowel disease (191) and colon carcinogenesis (348). These cells are activated by IL-23 and produce Th17 cytokines, including IL-17, IL-21, and IL-22 (159).

H. pylori-induced gastritis is driven by a variety of bacterial factors that stimulate epithelial cell, macrophage, and DC activation, as well as a Th1-predominant lymphocyte response; the role of the Th17 versus Th1 response is an area of intense investigation. Colonization of *H. pylori* can be abrogated by immunization with bacterial components such as urease (236), indicating activation of the adaptive response, but urease is also a major inducer of innate responses in monocytes and macrophages, stimulating cytokine and NO generation (118, 188, 189). Thus, determining whether the response of a particular cell type represents purely an innate or adaptive response is difficult, and the recognition that cells such as B cells can respond to *H. pylori* directly or via interaction with activated T cells illustrates the complexity of the immune response.

Immune Response to *H. pylori*

H. pylori induces both humoral and cellular immune responses. Local and systemic antibody responses have been demonstrated that include IgA, IgM, and IgG isotypes (67, 255, 349). Early studies with mouse models demonstrated that immunization with *H. pylori* antigens could induce protective immunity (194).

Although *H. pylori* proteins have been demonstrated in the lamina propria of the stomach (188), *H. pylori* has generally

been considered a noninvasive pathogen residing primarily in the extracellular mucus layer. However, several studies have demonstrated the ability of *H. pylori* to invade gastric epithelial cells both *in vitro* (12) and *in vivo*, in the stomachs of humans and monkeys (282) as well as in mice with atrophic gastritis (230). *H. pylori* bacteria have also been shown to be bound to erythrocytes within the microvessels of the lamina propria (21). Transmission electron microscopy and immunogold detection have been used to elegantly demonstrate that *H. pylori* cells are in direct contact with immune cells of the lamina propria in the majority of cases of gastritis and gastric cancer (208).

Macrophages. (i) Macrophage signaling of T cells. Macrophages are essential as innate responders to *H. pylori*-derived products and signals from epithelial cells that are in direct contact with the bacterium on the surface of the mucosa. Monocytes and macrophages are important coordinators of immune responses to pathogens, and in the case of *H. pylori*, they are likely activators, along with DCs, of adaptive immunity by producing factors, such as IL-12 (124, 201, 202), that stimulate Th1 cells, resulting in production of cytokines such as IFN-γ (124, 201, 202). The neutrophil-activating protein (NAP) of *H. pylori* contributes to Th1 polarization by stimulating both IL-12 and IL-23 secretion from neutrophils and monocytes (11). IL-12 production in the gastric mucosa is linked to the development of peptic ulcers in infection with *cagA*-positive *H. pylori* strains, most likely due to stimulation of Th1 responses (131). Macrophages are also involved in amplification of the inflammatory response by production of cytokines such as IL-1, TNF-α, and IL-6 (115, 127, 189). IL-6 activation has been linked to activation of TLR4, MAP kinase, and NF-κB signaling events (241).

IL-12 and IL-23 share the common p40 subunit, with a heterodimer of p19 and p40 constituting IL-23 and a heterodimer of p40 and p70 constituting IL-12. Notably, it was recently reported that in addition to increased expression of IL-12 p40 in human *H. pylori* gastritis, there is also increased immunostaining for IL-23 localized to macrophages in addition to epithelial cells (306). Therefore, additional studies are needed to clarify the role of IL-12 and IL-23 in the stimulation of T-cell responses.

(ii) Macrophages as effector cells. Macrophages constitute a potentially powerful line of defense against *H. pylori* through their own effector function, yet, intriguingly, these capabilities fail the host. One such pathway is the generation of nitric oxide (NO) derived from the enzyme inducible NO synthase (iNOS or NOS2), which has been shown to be upregulated by *H. pylori* in macrophages *in vitro* (42, 117, 118, 339) and *in vivo* (103, 192) (Fig. 3). Coculture studies demonstrate that *H. pylori* organisms can be eliminated by macrophages even when the bacteria are physically separated from these cells and that this antimicrobial defense is NO dependent (42, 117). The arginase enzyme possessed by *H. pylori*, encoded by the *rocF* gene, can compete sufficiently with macrophages for the iNOS substrate L-arginine (L-Arg) that host NO production is impaired, leading to enhanced survival of the bacterium through this mechanism (117). Moreover, this competition can deplete L-Arg sufficiently to impair the synthesis of iNOS protein, since its translation is highly dependent on L-Arg availability inside the

macrophage (46). Bacterial arginase serves to generate urea from L-Arg, which is then utilized by urease to synthesize ammonia that is required to neutralize gastric acid.

While the induction of iNOS in macrophages is termed classical activation of the M1 type, the alternative, M2 pathway involving the metabolism of L-Arg by arginase is also involved (Fig. 3). Exposure of macrophages to *H. pylori* products results in upregulation of the enzyme arginase II (Arg2) (116), which produces L-ornithine in addition to urea. Arginase induction exerts at least three potentially pathogenic roles. First, arginase depletes substrate availability for iNOS. In *H. pylori*-stimulated macrophages, iNOS protein translation is dependent on the level of L-Arg in culture medium and bacterial killing requires high levels of L-Arg (46). Consistent with this, increased iNOS translation and NO production occurred with inhibition of arginase or siRNA knockdown of Arg2 or in primary macrophages from Arg2$^{-/-}$ mice, while oral administration of an arginase inhibitor to *H. pylori*-infected mice increased iNOS protein expression and NO production by gastric macrophages (174). Second, Arg2 has a central role in inducing apoptosis of macrophages, which is dependent on the metabolism of its product, L-ornithine, into polyamines (116). Finally, the generation of ornithine by arginase results in increased substrate levels for the generation of polyamines by ornithine decarboxylase (ODC), which is also induced by *H. pylori* (48, 50, 116), and this results in inhibition of iNOS (42). Specifically, the polyamine spermine does not alter iNOS transcription, but instead, it blocks iNOS protein translation and NO production. Knockdown of ODC by RNA interference results in sufficient increases in iNOS protein expression and NO production that killing of *H. pylori* by macrophages can be enhanced significantly (42).

Inhibition of iNOS by spermine can also be attributed to blocking the uptake of L-Arg into macrophages by cationic amino acid transporter 2 (CAT2), required for iNOS translation, and depletion of spermine by the induction of the metabolic enzyme SMO facilitates iNOS protein synthesis and NO production (47). The biochemical pathways that limit NO production (summarized in Fig. 3) may exist to protect macrophages from the potential toxic effects of overproduction of NO in response to other pathogens, but in the case of *H. pylori*, the bacterium may usurp these host effectors to establish long-term colonization. These findings indicate that the dysfunction of the mucosal macrophage iNOS-derived NO synthesis plays a fundamental role in the persistence of the bacterium and, by definition, the risk for neoplastic transformation.

(iii) Macrophage apoptosis. Host inflammatory responses are enhanced by macrophage activation, but apoptosis of macrophages can have profoundly deleterious consequences. The release of cytokines from dying cells may be important, as originally established for *Shigella* infection (365), and apoptotic cells stimulate infiltration of neutrophils to engulf cellular debris, leading to potentiation of inflammation and increased oxidative stress from oxyradicals released by activated neutrophils. Also important is the net effect of a loss of host defense with the depletion of these effector cells. The generation of polyamines is directly involved in macrophage apoptosis (Fig. 4). The production of putrescine by ODC results in the generation of spermidine and spermine by constitutive synthase enzymes. Spermine is then back-converted by the enzyme SMO to spermidine, with the by-product of this metabolism
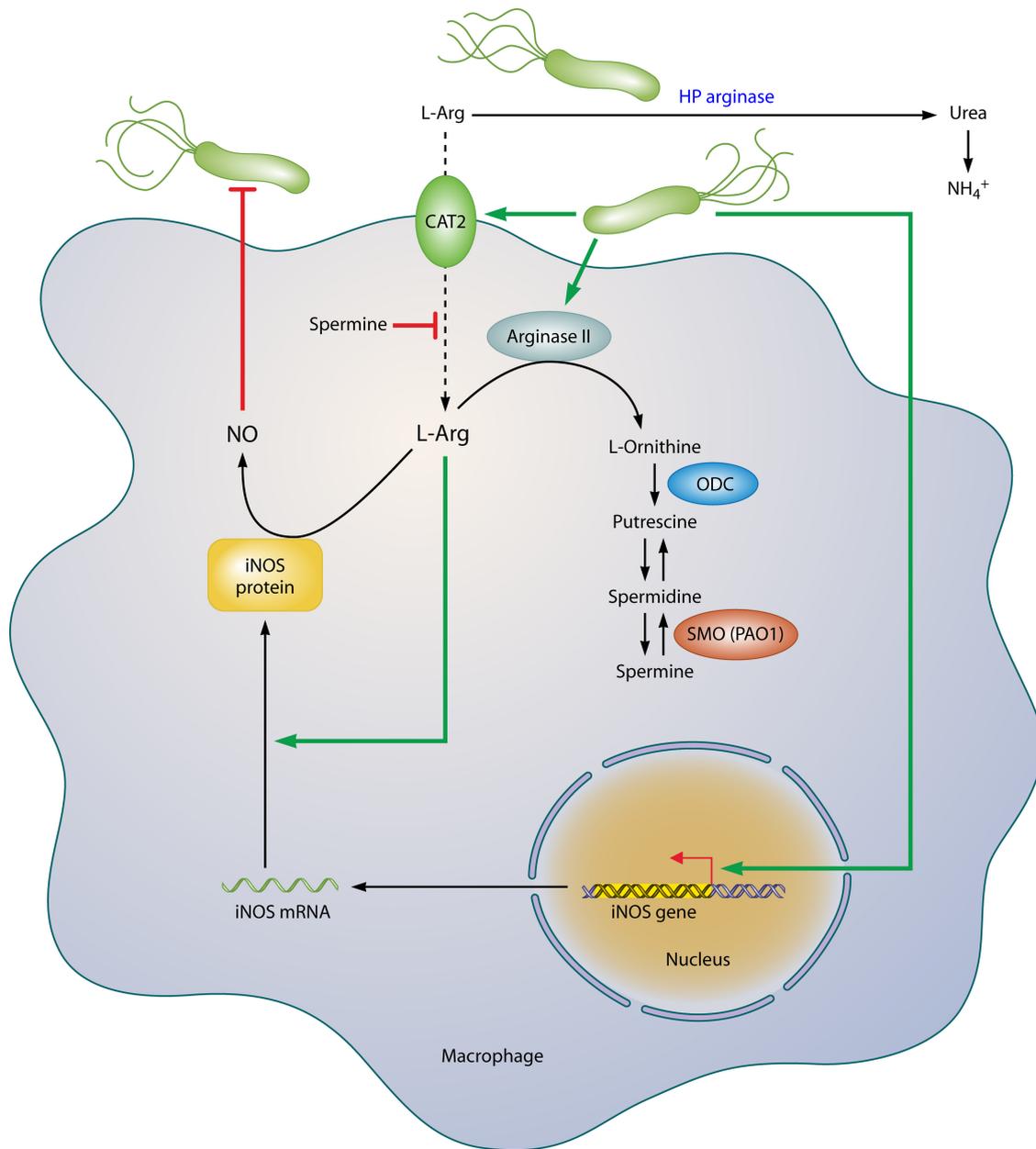
FIG. 3. Pathways involved in regulation of macrophage iNOS synthesis and NO production in response to *H. pylori*. The translation of iNOS protein depends on the availability of L-arginine (L-Arg). Pathogenic mechanisms that inhibit L-Arg availability for iNOS include (i) the consumption of extracellular L-Arg by *H. pylori* itself, through its bacterial arginase activity; (ii) the upregulation of macrophage arginase II, which depletes intracellular L-Arg; and (iii) induction of ODC that generates the polyamine spermine, which blocks uptake of L-Arg into macrophages by CAT2. The resulting effect is limitation of iNOS protein synthesis and NO production, despite high levels of iNOS mRNA. Arginase and ODC are novel targets for therapeutic intervention to enhance antimicrobial NO production and hence reduce persistent colonization that leads to chronic inflammation and cancer risk.

being hydrogen peroxide (H_2O_2) (48). SMO is upregulated by *H. pylori* in macrophages, and its inhibition by the pharmacologic inhibitor MDL 72527 or by RNA interference prevents the generation of H_2O_2 and the intrinsic pathway of apoptosis in macrophages, since both mitochondrial membrane depolarization and caspase-3 activation are abrogated (48). *H. pylori* also upregulates expression and nuclear translocation of c-Myc, and the binding of this transcription factor to the 5′-untranslated region (5′-UTR) of the ODC promoter mediates ODC transcription and associated apoptosis (50).

(iv) Avoidance of phagocytosis by macrophages. Another potential factor that may contribute to the failure of the innate immune response to eliminate *H. pylori* is that the bacterium can evade effective phagocytosis by macrophages (9, 364). Although *H. pylori* can be internalized by macrophages, into phagosomes, these phagosomes fuse and form "magasomes" containing large numbers of live bacteria. Additionally, *H. pylori* strains containing the *cag* PAI and producing a functional VacA protein prevent the fusion of phagosomes with lysosomes needed for bacterial killing, and disruption of
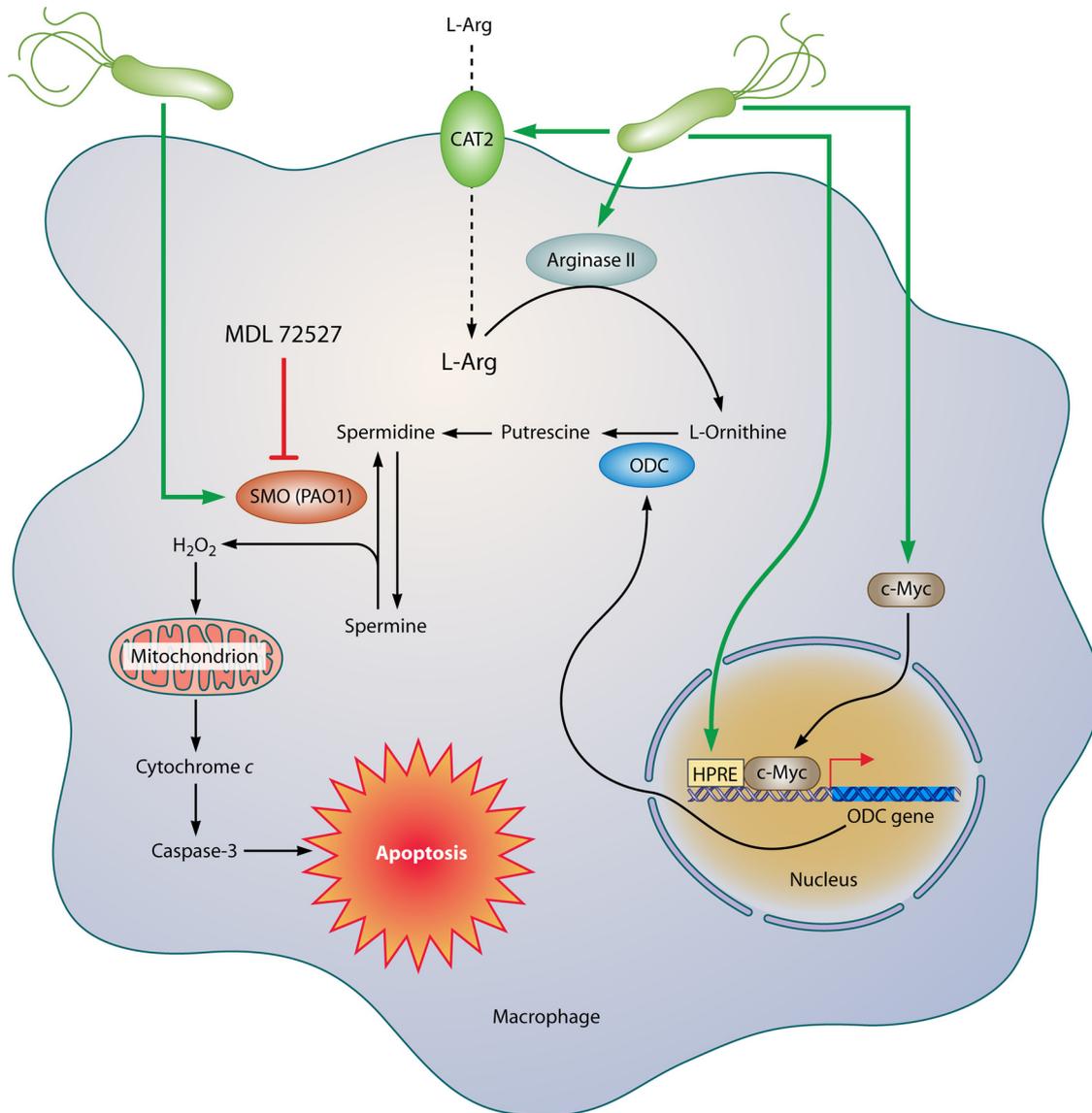
FIG. 4. Mechanism of macrophage apoptosis caused by *H. pylori*. This pathway is dependent on the activities of the enzymes arginase II, ODC, and SMO. Induction of arginase II enhances synthesis of L-ornithine, which is converted into polyamines by ODC via a process that requires both *H. pylori* activation of the ODC promoter and c-Myc as a transcriptional enhancer. Production of the polyamine spermine provides a substrate for SMO, which is also upregulated by *H. pylori*. SMO generates H_2O_2, which causes mitochondrial membrane depolarization, cytochrome *c* release from mitochondria to the cytosol, and caspase-3 activation, followed by apoptosis. Induction of macrophage apoptosis leads to impairment of mucosal immunity to *H. pylori*, chronic inflammation, and cancer risk (48, 50, 116).

phagosome maturation is lost when cells are exposed to an isogenic *vacA* mutant strain (364).

DCs. DCs represent a critical bridge between the innate and adaptive immune responses. DCs have been identified as primary responders to stimuli that include bacterial products (251), and they serve as antigen-presenting cells (APCs) (272). DCs can penetrate epithelial monolayers *in vitro* and the intestinal epithelial barrier *in vivo*, and they take up bacteria directly (51, 221, 257). Disruption of the epithelial apical-junctional complex by *H. pylori* (13, 93) could facilitate both luminal and subepithelial interaction of DCs with *H. pylori* and antigens shed by the bacterium. After activation of TLRs, DCs in turn activate T cells in different ways, being capable of inducing either a Th1 or Th2/

regulatory T-cell (Treg) response by generation of IL-12 or IL-10 (30). DCs derived from human peripheral blood mononuclear cells have been shown to produce IL-12 and IL-10 when stimulated with *H. pylori ex vivo* (121, 162). Pulsing of human DCs with intact *H. pylori* and bacterial membrane preparations results in DC maturation. Coculture of activated DCs with NK cells results in secretion of TNF-α and IFN-γ, and coculture with naïve T cells results in TNF-α, IFN-γ, and IL-2 secretion, indicative of NK and Th1 effector responses (125).

H. pylori outer membrane proteins such as Omp18 and HpaA have been reported to induce the maturation and antigen presentation of DCs (324). In human blood monocyte-derived DCs, activation and maturation of DCs occur indepen-

dently of the presence of the *cag* PAI and *vacA* genotype, and activation of cytokine production does not require live bacteria (163). However, IL-12 responses are attenuated with inhibition of bacterial internalization (125, 163), indicating that phagocytosis of intact *H. pylori* by DCs may activate intracellular receptors. DCs interact with *H. pylori* by binding of glycoconjugate carbohydrate structures to DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN/CD209). The identification of DC-SIGN as a novel receptor for *H. pylori* may be critical to understanding the shifting of T-cell responses in favor of persistence of the infection (17). Specifically, Lewis antigen expression by *H. pylori* LPS can block Th1 responses by binding to DC-SIGN on DCs, thus representing a form of immunosuppression (17).

Stimulation of mouse bone marrow-derived DCs with *H. pylori* results in phagocytosis of bacteria and expression of the proinflammatory cytokines IL-1α, IL-1β, and IL-6; however, this results in only modest IL-12 expression and diminished activation of splenocyte IFN-γ secretion and cellular proliferation compared to those induced by *Acinetobacter lwoffii* (151), another pathogen that causes gastritis in mice (361). DCs activated with *H. pylori* for 48 h exhibited an attenuated ability to induce IFN-γ production upon coculture with naïve T cells compared to DCs pulsed with *H. pylori* for only 8 h, and there was a similar loss of response to CD40 ligation (206). This suggests that chronic exposure of DCs to *H. pylori* results in a loss of ability to induce a Th1 response that could contribute to the persistence of the infection.

It has been reported that DCs are recruited into the stomach as early as 6 h postinoculation (151). Recently, two-photon microscopy revealed that DCs extend dendrites into the gastric lumen after *H. pylori* infection of the mouse stomach (152). *Ex vivo* activation of DCs by *H. pylori* and adoptive transfer result in a skewing of the Th17/Treg balance toward Tregs, with experimental enhancement of immunity accomplished by depletion of Tregs and enhancement of the Th17 response, indicating that the DC response to *H. pylori* may contribute to bacterial immune evasion (152) and thus lead to chronic inflammation and cancer risk.

T cells. Early studies established the concepts that gastric lymphocytes from *H. pylori*-infected patients have increased numbers of IFN-γ-producing T cells, consistent with a Th1 cytokine response (29, 155), and that *H. pylori*-specific T-cell clones derived from gastric mucosa exhibit a Th1 profile in patients with peptic ulcer disease (70). Mucosal T cells in *H. pylori* infection produce abundant levels of the Th1 cytokines IFN-γ and IL-2 and low levels of the Th2 cytokines IL-4 and IL-5 (29, 155). IL-12 production derived from monocytes, macrophages, or DCs is important in the induction of Th1 lymphocyte responses (121, 124, 125, 162, 201, 202), and the role of gastric epithelial cells as antigen-presenting cells in the activation of CD4$^+$ Th cells has also been demonstrated (359).

IFN-γ$^{-/-}$ mice have decreased levels of gastric inflammation (3), and SCID mice lacking T and B cells infected with *H. pylori* require adoptive transfer of CD4$^+$ T cells for the development of gastritis (77); such studies have shown that an insufficient Th1 response is associated with increased bacterial colonization (3, 77). These studies suggest that the development of a strong Th1 response can lead to an attenuation of *H. pylori* colonization (77, 78). However, there is also evidence

that adoptive transfer into SCID mice of CD4$^+$ T cells from T-bet$^{-/-}$ mice, which do not exhibit IFN-γ production and Th1 differentiation, still results in gastritis (76).

IL-17 has been linked to chemokine-mediated neutrophil infiltration (97), and IL-17 levels are increased in infected human (183) and mouse (8a) gastric tissues. Immunization of mice with *H. pylori* lysate enhances IL-17 expression in the gastric mucosa and in CD4$^+$ T cells isolated from spleens and cocultured with *H. pylori*-pulsed DCs or macrophages; these findings were associated with increased gastric inflammation and decreased colonization (71). These data suggest that a defective IL-17/Th17 response contributes to chronic persistence of the bacterium. Another mechanism of immune dysfunction has been demonstrated by the recent report that VacA can exert immunosuppressive effects on T cells by binding to the β2 integrin receptor subunit (CD18) and utilizing integrin receptors to cause cellular vacuolization (284).

Additional investigations have implicated Tregs in the pathogenesis of *H. pylori* infection. Circulating memory T cells from *H. pylori*-infected humans have less proliferation and IFN-γ production in response to *H. pylori*-pulsed DCs than do T cells from naïve donors, and this defect can be abrogated by depletion of CD4$^+$ CD25^{high} regulatory T cells, indicating that *H. pylori*-specific Tregs suppress memory T-cell responses and contribute to the persistence of the infection (182). *H. pylori*-infected individuals have increased levels of CD4$^+$ CD25^{high} T cells in the gastric and duodenal mucosa that express *FOXP3*, a gene involved in the development of Tregs, and high levels of the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) protein (181).

In athymic nude mice lacking T cells, reconstitution with lymph node cells depleted of CD25$^+$ T cells resulted in a significant reduction in *H. pylori* colonization and in increased gastritis, infiltrating CD4$^+$ T cells, and production of IFN-γ compared to those in mice receiving nondepleted lymph node T cells (254). BALB/c mice infected with *H. pylori* or *H. felis* and treated with anti-CD25 antibody to deplete Tregs developed a Th2 response characterized by serum IgG1 antibodies and production of IL-4 and IL-5 in paragastric lymph node-derived T cells (153). These data suggest that the Treg response can potentially act to enhance Th1 commitment, but this study did not detect any difference in colonization or severity of gastritis (153). The anti-CD25 monoclonal antibody PC61 depletes Foxp3$^+$ Treg cells and resulted in increased *H. pylori* gastritis severity, gastric cytokine levels, and serum IgG1 and IgG2c levels and a concomitant decrease in bacterial colonization in C57BL/6 mice (253). In conjunction with findings that *H. pylori*-infected patients express increased levels of *FOXP3* mRNA and protein in gastric lymphocytes (253), this study suggests that the induction of a Treg response contributes to establishing an equilibrium between host and bacterium, allowing *H. pylori* to survive, but also preventing the risk of destructive inflammation.

Activation of T cells by specific antigens involves expression of costimulatory molecules, and CTLA-4 inhibits this process. In the case of *H. pylori* infection, functional inactivation of CD4$^+$ T cells recruited to the gastric mucosa may be related to expression of CTLA-4 on the T-cell surface and prevention of costimulation when APCs engage T-cell receptors (16). Blockade of CTLA-4 resulted in increased T-cell activation *in vitro*

and *in vivo* and in decreased colonization in *H. pylori*-infected mice, suggesting that there is an induction of anergy in CD4$^+$ gastric T cells (16). *H. pylori* can inhibit lymphocyte proliferation (111, 202), an effect attributed to a downregulatory effect of *H. pylori* VacA on the activation and nuclear translocation of the transcription factor nuclear factor of activated T cells (NFAT) (35, 111). VacA has also been shown to activate MAP kinase signaling that results in activation of the GTPase Rac, leading to disruption of the cytoskeleton due to actin rearrangement (35).

Collectively, there are numerous features of T-cell function that are altered in a deleterious manner by *H. pylori* infection. However, exciting developments related to understanding the role of Th17 cells and Tregs may lead to new therapeutic avenues to reduce chronic inflammation and cancer risk.

B cells. There are ample data indicating that B cells also contribute to *H. pylori* pathogenesis. In B-cell-deficient (μMT) mice infected with *H. pylori*, a 2-log reduction in *H. pylori* colonization was reported that was associated with increased gastric inflammation and infiltration of CD4$^+$ T cells (4). While IgG and IgA responses to *H. pylori* in the serum and gastric mucosa may be involved in protective immunity, this study and another (5) suggest that B-cell-mediated antibody responses may be counterproductive. A major focus of investigation has been related to the development of gastric MALT lymphoma, which arises from activated B cells. Naïve mouse splenocytes exposed to *H. pylori* are protected from spontaneous apoptosis and exhibit proliferation in response to low, but not high, multiplicities of infection, and the responding cells are derived from B-cell populations (41). Furthermore, chronic infection with *H. pylori* protects splenic B cells from apoptosis, indicating a B-cell activation/survival phenotype that may have implications for MALT lymphoma (41). In addition to producing antigen-specific antibodies, B cells have also been shown to produce autoreactive antibodies that may be pathogenic (351). The role of T-cell–B-cell interactions in the pathology of the immune response is a fertile area of future investigation.

Inflammation-mediated migration of peripheral cells. Chronic inflammation that develops in response to *H. pylori* undoubtedly contributes to transformation. Studies of mice infected with *H. felis* have demonstrated that bone marrow-derived cells (BMDC) home to and engraft in sites of chronic inflammation, particularly within foci where tissue injury induces excessive apoptosis, which overwhelms the population of endogenous stem cells (140). Within the inflammatory environment of the infected stomach, BMDC degenerate into adenocarcinoma, suggesting that gastric epithelial carcinomas can originate from bone marrow-derived sources (140).

Apical-Junctional Complexes

Gastric mucosal barrier function is essential for preventing potentially harmful elements present in the gastric lumen from gaining access to the gastric mucosa. Barrier function is compromised in *H. pylori*-induced gastritis (298) and in intestinal epithelial cells infected with *H. pylori* (93). The apical-junctional complex, which controls barrier function, is composed of tight junctions, which are located at the most apical region of cells, and adherens junctions, which are located immediately below (Fig. 5).

Tight junctions. Tight junction complexes are dynamic structures composed of integral membrane proteins, such as occludin, junctional adhesion molecules (JAMs), and the claudins, which are a family of 24 tetraspan transmembrane proteins (318), as well as membrane-associated proteins such as ZO-1. These components play critical roles in maintenance of barrier function, cell polarity, and intercellular adhesion. Disruption of the tight junction complex is associated with a variety of human diseases, including cancers of the gastrointestinal tract (318), and specific *H. pylori* constituents have also been reported to be involved in dysregulation of tight junctions.

The majority of *H. pylori* organisms are thought to be free-living within the protective mucous layer of the stomach; however, approximately 20% of bacteria attach to gastric epithelial cells. *H. pylori* preferentially adheres to gastric epithelial cells in close proximity to tight junctions (13, 129) and can alter localization of the component proteins that constitute apical-junctional complexes (13, 93, 164, 345). *H. pylori* has been detected within intraepithelial intercellular spaces directly beneath tight junctions, leading investigators to hypothesize that the tight junction may be a portal of entry for the bacterium (218), which allows the bacterium access to essential nutrients from epithelial cells to support its growth (307). Recently, viable *H. pylori* cells have also been identified within the lamina propria, gastric lymph nodes, and intracellular canaliculi of parietal cells (21, 144).

A variant of *H. pylori* that was cell adapted for increased adhesion to MDCK cells and that more efficiently translocated CagA into MDCK cells was found to modify ZO-1 expression at the tight junction in polarized MDCK cells and to recruit ZO-1 away from the tight junction to sites of bacterial attachment, which requires translocation of CagA (13). Ectopic expression of CagA in polarized MDCK cells causes a loss of apicobasal polarity, which is associated with an invasive cellular phenotype, and increased tight junction permeability (27). Similar to the case in MDCK cells, coculture of primary human epithelial cells with *H. pylori* altered the distribution of ZO-1 and induced an accumulation of ZO-1 in the cytoplasm that colocalized with CagA. However, the precise role of CagA in this model remains to be determined fully (164).

H. pylori replicates on the surfaces of MDCK cells, and cell polarity is an important factor for bacterial survival. CagA is required for *H. pylori* to disrupt MDCK cell polarity (307), and this allows *H. pylori* to potentially gain access to nutrients on the basolateral membrane that are required for replication. However, CagA is not required for replication on nonpolarized cells, where free mixing of molecules from the apical and lateral membranes occurs, and therefore no requirement exists for bacteria to acquire nutrients from within cells (307).

CagA specifically associates with PAR1b/MARK2, one of the four members of the PAR1 family of kinases (269). PAR1b is located on the basolateral surface membrane and has an essential role in maintaining epithelial cell polarity (269, 362). In a tyrosine phosphorylation-independent manner, CagA inhibits PAR1 kinase activity, and this prevents phosphorylation of PAR1, leading to its dissociation from the membrane, which causes defects in epithelial cell polarity and disruption of tight
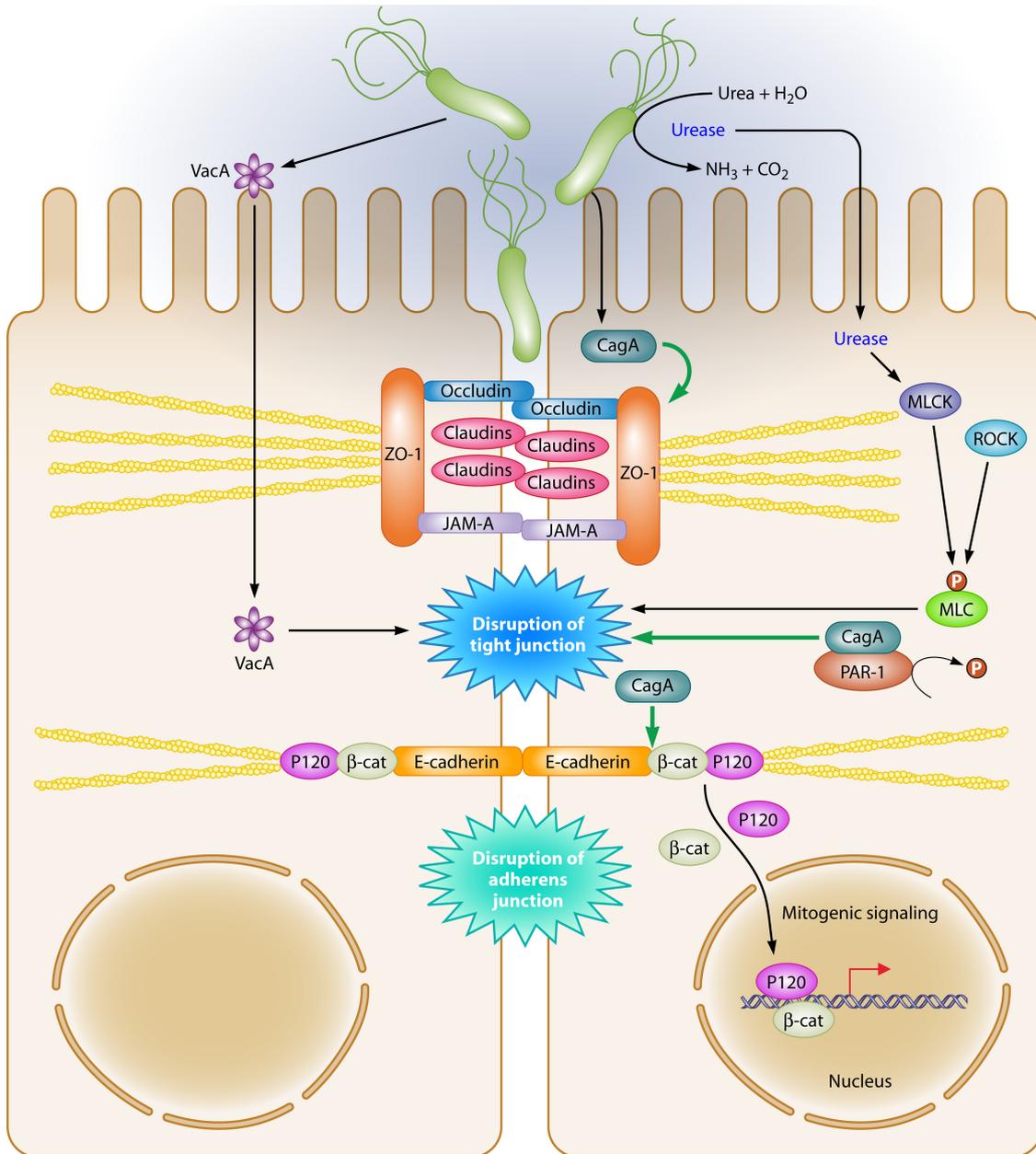
FIG. 5. Dysregulation of the apical-junctional complex by *H. pylori*. *H. pylori* preferentially adheres to the apical-junctional complex of epithelial cells and alters localization of apical-junctional component proteins, disrupts epithelial barrier function, cell adhesion, and cell polarity, and induces an invasive phenotype. Translocated CagA interacts with PAR1, preventing phosphorylation of PAR1 by blocking PAR1 kinase activity, which culminates in disruption of the tight junction. In addition, functional urease activity can disrupt the tight junction via a mechanism involving MLC phosphorylation, which can be regulated by MLCK and Rho kinase. VacA can also increase tight junction permeability to low-molecular-weight molecules and ions.

junctions (269) (Fig. 5). The PAR1b-binding region of CagA has been identified as the 16-amino-acid CagA sequence also known as the CagA multimerization (CM) sequence, which is involved in CagA dimerization (256). Interestingly, the CM sequence of CagA proteins isolated from East Asian *H. pylori* strains binds PAR1b more strongly than the CM sequence of proteins isolated from Western strains of *H. pylori* (180). Within Western *H. pylori* strains, the number of CM repeats is directly proportional to the ability of CagA to bind to PAR1b (180). The magnitude of tight junction disruption is propor-

tional to the level of PAR1b-binding activity of CagA (180). CagA-mediated inhibition of PAR1 kinase also contributes to the hummingbird phenotype by perturbing microtubules and nonmuscle myosin II (179). The mechanism by which CagA inhibits PAR1/MARK enzymes was recently determined using crystallography. CagA uses the initial 14 amino acids of the CM motif to bind to the MARK2 substrate binding site, thereby mimicking a host cell substrate that can inhibit a kinase and exert biological effects on the host cell (219).

The ability of CagA to disrupt tight junctions has not been

demonstrated in all studies, which may be due to the use of different bacterial strains and/or model systems. Although *H. pylori* strain SS1 is CagA$^+$, it lacks a functional type IV secretion system and cannot inject CagA into epithelial cells (65). Interestingly, this strain induces an inflammatory reaction and disrupts barrier function in the gastric mucosa (93, 301). In a CagA- and VacA-independent manner, *H. pylori* has also been shown to decrease expression of claudin-4 and claudin-5 and to activate myosin light chain kinase (MLCK), which subsequently disrupts barrier function (93).

Occludin is also implicated in regulation of gastric barrier function (49) and is linked directly to the actin cytoskeleton via its C terminus (340), and indirectly through its interactions with ZO-1 (89). Expression of occludin at the level of the tight junction is disrupted by *H. pylori* (93, 301, 345), which leads to gastric epithelial barrier dysfunction. This requires functional urease activity and is independent of CagA, VacA, or a functional *cag* PAI. Disruption of barrier function also involves MLCK-dependent phosphorylation of the myosin II regulatory light chain (MLC) (345). Ammonium produced by *H. pylori* similarly disrupts barrier function and leads to the generation of a 42-kDa truncated form of occludin in Caco-2 cells (184). In a mechanism independent of CagA, the addition of purified VacA to MDCK cells increases tight junction permeability to low-molecular-weight molecules and ions (Fig. 5). Interestingly, this increase in permeability is not associated with a redistribution or alteration in the total amount of the tight junction proteins ZO-1 and occludin (238).

Adherens junctions. Adherens junctions are required for cell-to-cell adhesion and are composed of cadherins, p120 catenin (p120), β-catenin, and α-catenin. The cadherins are a family of transmembrane proteins that form homodimers with cadherin molecules on adjacent cells. E-cadherin acts as a cell-cell adhesion molecule in epithelial tissues, and its turnover is regulated by binding of p120 to the cadherin juxtamembrane domain. The cytoplasmic domain of E-cadherin interacts directly with β-catenin and p120, and these molecules, in turn, interact with the actin cytoskeleton. Loss of E-cadherin function is associated with gastric cancer, and *H. pylori* infection induces E-cadherin gene promoter methylation, leading to a reduction in E-cadherin expression (44, 172, 246); also, eradication of *H. pylori* reduces E-cadherin promoter hypermethylation (45, 172, 246). Translocated CagA physically interacts with E-cadherin, leading to the destabilization of the E-cadherin–β-catenin complex and to accumulation of cytoplasmic and nuclear β-catenin, which then transactivates β-catenin-dependent genes that may promote carcinogenesis (212, 233). While *H. pylori* disrupts the apical junction, it does not alter expression of E-cadherin but, instead, induces translocation of E-cadherin, β-catenin, and p120 from the membrane into the cytoplasm of epithelial cells (54, 227, 303, 333) (Fig. 5). Recent work suggests that CagA directly interacts with E-cadherin and p120 and forms a complex composed of c-Met, E-cadherin, and p120, which prevents tyrosine phosphorylation of c-Met and p120 and suppresses cell-invasive phenotypes (233).

In addition to the membrane, β-catenin is also present within the cytoplasm, where it is phosphorylated by GSK-3β, within a multiprotein inhibitory complex that includes the adenomatous polyposis coli tumor suppressor protein. This complex targets β-catenin for ubiquitination and subsequent degradation (309). Increased expression of β-catenin, mutations within the adenomatous polyposis coli protein, and inhibition of GSK-3β are frequently observed in gastric cancer specimens and function to stabilize β-catenin in the cytoplasm (314). *H. pylori* can activate β-catenin through PI3K-dependent inactivation of GSK-3β (216, 302) and can also interact with membrane-associated β-catenin via CagA to activate β-catenin signaling and promote mitogenic signaling (168, 212). In a gerbil model of infection, a carcinogenic strain of *H. pylori* increased nuclear accumulation of β-catenin, which is consistent with observations in human gastric biopsy specimens, where increased nuclear β-catenin was seen for patients infected with *H. pylori cag$^+$* strains compared to that for persons infected with *H. pylori cag*-deficient mutant strains or in uninfected gastric biopsy specimens (101).

In addition to downregulation of E-cadherin, reduced membrane expression or aberrant localization of p120 to the cytosol or nucleus is frequently observed in gastric cancer (145, 154). Loss of E-cadherin or overexpression of p120 *in vitro* results in mislocalization of p120 to the cytoplasm (258), where it can promote motility and metastasis via interactions between p120 and Rho GTPases (14, 120, 223). In normal cells, p120 expression is typically low in cell nuclei; however, expression is increased within the nuclei of tumor cells (197, 273, 335). Recently, *H. pylori* infection has been associated with mislocalization of p120 to the nucleus in human gastric epithelia and infected murine primary gastric epithelial cells (Fig. 5) (164, 227). Mechanistically, nuclear p120 relieves transcriptional repression of *mmp-7* via interacting with Kaiso. MMP-7 is a matrix metalloproteinase implicated in gastric tumorigenesis (discussed in detail below), and aberrant activation of this molecule likely represents another mechanism through which *H. pylori* may lower the threshold for developing gastric cancer (227).

Alterations in Cellular Turnover That Predispose Individuals to *H. pylori*-Induced Malignant Transformation

Maintenance of tissue integrity requires that enhanced cell proliferation be accompanied by increased rates of cell loss. In chronic *H. pylori* infection, there is a lack of epithelial necrosis (210), suggesting that other forms of cellular demise may be affected. Apoptosis is a form of programmed cell death consisting of a tightly regulated series of energy-dependent molecular events (322), and *H. pylori* stimulates apoptosis *in vitro* via multiple mechanisms (63, 88, 138, 139, 147, 266, 327, 330). Transient expression of VacA in HeLa cells induces the release of cytochrome *c* from mitochondria, leading to activation of caspase-3 and induction of apoptosis. These findings were confirmed by studies using wild-type and *vacA* mutant *H. pylori* strains (63, 166), as s1/m1 types of VacA induced higher levels of apoptosis than s2/m1 toxins or VacA mutants lacking the hydrophobic amino-terminal region.

In contrast to the case for cell culture, however, *H. pylori* has been associated with increased (148, 192, 210, 266) or decreased (136) levels of apoptosis *in vivo*, and within a particular patient population, substantial heterogeneity exists among apoptosis scores (148, 192, 210, 266). One explanation for

these discordant results is that differences in levels of apoptosis among *H. pylori*-infected persons may result from strain-dependent variations in VacA structure. However, *H. pylori* was recently shown to inhibit gastric epithelial cell apoptosis in Mongolian gerbils (204), and this was associated with epithelial cell hyperplasia and persistent bacterial colonization of the stomach. Suppression of apoptosis *in vivo* was mediated by CagA, which stimulated the prosurvival MAP kinase ERK1/2 and the antiapoptotic protein MCL1 within gastric pits. Thus, CagA may counteract the effects of VacA and activate cell survival and antiapoptotic pathways to overcome self-renewal of the gastric epithelium and to help sustain *H. pylori* infection (204).

Another possibility for observed differences in the degree of cell death within inflamed gastric tissue is that certain *H. pylori*-induced host effectors may differentially regulate apoptosis depending on the particular microenvironment. MMP-7 is a matrix metalloproteinase with matrix-degrading and tumor-initiating properties that is expressed and secreted by epithelial cells (57). Overexpression of MMP-7 occurs in premalignant and malignant lesions in the stomach (2, 135, 137, 199, 270, 283, 355), and genetic polymorphisms linked to increased MMP-7 expression are associated with *H. pylori* infection status, gastric ulceration (a precursor for gastric cancer), and tumor-related survival among gastric cancer patients (130, 165). In mice, overexpression of MMP-7 leads to hyperproliferation and increased cancer susceptibility (267), while cell lines that overexpress MMP-7 develop enhanced tumorigenic potential (341). Conversely, MMP-7 deficiency attenuates tumorigenesis in mice with a genetic predisposition for intestinal adenocarcinoma (338).

Colonization by *H. pylori cag*+ strains leads to enhanced levels of MMP-7 within human gastric mucosa (69), and *in vitro*, *cag*+ isolates selectively induce MMP-7 in epithelial cells, dependent upon activation of ERK1/2 by specific components within the *cag* island (69). In addition to matrix degradation, MMP-7 alters epithelial proliferation and apoptosis. MMP-7-treated myofibroblasts increase gastric epithelial cell proliferation *in vitro* via insulin growth factor II (IGF-II) (198, 344). Short-term treatment of epithelial cells with MMP-7 cleaves membrane-bound FasL, which then induces apoptosis (95, 250). However, chronic solubilization of FasL by MMP-7 selects for a population of cells with reduced sensitivity to apoptotic stimuli (95).

A specific mechanism through which MMP-7 inhibits apoptosis is by cleaving proheparin-binding epidermal growth factor (pro-HB-EGF) to active HB-EGF, which promotes cell survival by EGFR (360). Consistent with these findings, *H. pylori* transactivates EGFR via activation of HB-EGF (158). *H. pylori* infection, gastric epithelial hyperplasia, and gastric atrophy are associated with dysregulation of EGFR and/or cognate ligands in human, animal, and cell culture models (262, 268, 328, 343, 358). Also, *H. pylori*-mediated activation of EGFR is dependent upon genes within the *H. pylori cag* pathogenicity island. Thus, chronic stimulation of MMP-7 by *H. pylori in vivo* may select for a population of hyperproliferating cells with reduced sensitivity to apoptosis, thereby contributing to increased carcinogenic risk.

ENVIRONMENTAL FACTORS

Role of Salt as a Risk Factor for Gastric Adenocarcinoma

The risk of gastric carcinoma is influenced not only by *H. pylori* strain characteristics and host genetic determinants but also by environmental elements. One factor that has uniformly been associated with an increased risk of gastric cancer is high dietary salt intake (56, 104, 313). This association has been detected in prospective studies, case-control studies, and a study that compared urinary salt excretion with gastric morbidity rates. A prospective study of a Japanese population and a case-control study in South Korea each reported that *H. pylori*-infected subjects consuming a high-salt diet had an increased risk of gastric cancer compared to *H. pylori*-infected subjects who consumed lower levels of salt (169, 286), while another study reported a positive correlation between the prevalence of *H. pylori* infection and levels of dietary salt intake (32).

Investigations using Mongolian gerbils have shown that the presence of *H. pylori* and use of a high-salt diet exert synergistic effects on the development of premalignant lesions or gastric cancer (108, 156, 297, 363). Specifically, Gamboa-Dominguez et al. reported that *H. pylori*-infected gerbils fed a high-salt diet developed more severe gastric inflammation and higher levels of gastric epithelial proliferation than those in *H. pylori*-infected gerbils consuming a regular diet (108). Similarly, Kato et al. and Nozaki et al. studied *H. pylori*-infected gerbils treated with a carcinogen (*N*-methyl-*N*-nitrosourea [MNU]) and reported that animals fed a high-salt diet had a higher incidence of gastric cancer than animals fed a regular diet (156, 224). Sun et al. reported that levels of three proinflammatory cytokines (IL-1, IL-6, and TNF-α) were higher in *H. pylori*-infected gerbils fed a high-salt diet than in infected gerbils fed a regular diet (297). Similarly, hyperosmotic stress has been reported to enhance *H. pylori*-induced expression of IL-1β by gastric epithelial cells *in vitro* (363).

The potential synergistic effects of salt and *H. pylori* infection on gastric inflammation and damage have also been studied in mice, although not to the same extent as in gerbils. One study reported that in comparison to a low-salt diet, high salt intake enhanced *H. pylori* colonization in C57BL/6 mice and resulted in increased gastric epithelial cell proliferation (99). However, other studies have not detected any effects of a high-salt diet in various mouse models (171, 261, 312).

The mechanisms by which salt increases the risk of gastric cancer in humans are incompletely understood. One possibility is that salt may have direct effects on the gastric epithelium that lower the threshold for malignant transformation. Another possibility is that salt damages the gastric mucosa, thereby allowing entry of carcinogens into gastric tissue. Upregulated production of cytokines in response to a high-salt diet may also be contributory. Finally, an intriguing possibility raised by recent observations is that high salt concentrations could potentially modulate gene expression in *H. pylori*. Consistent with this hypothesis, two groups have now reported that high salt concentrations result in increased expression of *H. pylori* virulence factors, including CagA (109, 177), providing important insights into mechanisms through which high-salt

diets increase the risk for gastric cancer among subjects colonized with disease-associated strains.

Helminth Infection

Animal models and small human studies have suggested that coinfection with helminths may have an impact on the outcome of *H. pylori* infection. Investigations using mice have shown that concurrent infection with helminths can reduce the severity of *Helicobacter*-induced gastritis. This was coupled with a reduced Th1 response concomitant with higher levels of Th2 cytokines (98). One study of a Chinese population reported that concurrent helminth infections modified serological IgG responses to *H. pylori* and that this was associated with a decreased risk of developing *H. pylori*-induced atrophy, as determined by the pepsinogen I/II ratio (75). Similar results were reported in a study of Colombian children, where a higher Th2-associated IgG1 response was reported for children infected with both helminths and *H. pylori* and living in a coastal region where the incidence of gastric cancer is low (334). This represents an intriguing area of research with potential for future studies focused on *H. pylori* pathogenesis.

Dietary Antioxidants

There has been extensive research conducted into the protective role of antioxidants found in food against the development of gastric cancer (291). However, less is known about interactions between *H. pylori* infection and dietary factors in gastric cancer. A randomized trial conducted on a population at high risk of developing gastric cancer suggested that eradication of *H. pylori* in conjunction with dietary supplementation with vitamin C and β-carotene increased the regression of preneoplastic lesions at 6 years of follow-up (58). However, following a further 6 years without dietary supplementation, the preventative effects of vitamin C and β-carotene disappeared (200). A population-based case-control study in Sweden suggested that a high intake of dietary vitamin C and β-carotene may lower the risk for developing gastric cancer in *H. pylori*-infected individuals (80). Concordant with the work of Ekstrom et al. (80), a case-control study in Hawaii suggested that vegetable intake among individuals infected with *H. pylori* provided some protection against developing gastric cancer (84). Conversely, a prospective study involving 10 European countries suggested that there was no significant interaction between *H. pylori* infection, plasma vitamin C levels, and the risk of developing gastric cancer (146). Further studies will be needed to determine whether antioxidants are protective against gastric cancer among *H. pylori*-infected patients.

Cigarette Smoking

Several studies have demonstrated that cigarette smoking increases the *H. pylori*-associated risk of developing gastric cancer. A population-based prospective study of Japanese males suggested that cigarette smoking and *H. pylori* are risk factors for gastric cancer and that the combination of both factors further increased the risk of developing gastric cancer (285), and similar results were obtained in a Swedish study (288). Cigarette smoking in combination with infection by *H.*

pylori CagA[+] strains was also found to increase the risk of developing gastric cancer in a German population-based case-control study (39). However, while a case-control study in Japan reported that *H. pylori* and cigarette smoking were individually associated with gastric cancer, there was no significant association between *H. pylori* and cigarette smoking (186). A population-based case-control study in Los Angeles County also reported no statistically significant association between gastric cancer risk and *H. pylori*-infected smokers, although a trend toward increased risk in current smokers was observed (346). Taking these data together, it appears that there is a positive association between *H. pylori* infection and smoking for the risk of developing gastric cancer, and it is likely that the combined effects of all environmental factors play a role in the risk of developing gastric cancer among *H. pylori*-infected individuals.

CONCLUSIONS

Gastric cancer is a highly lethal disease, and establishment of *H. pylori* as a risk factor for this malignancy permits an approach to identify persons at increased risk; however, infection with this organism is extremely common, and most colonized persons never develop cancer. Thus, techniques to identify high-risk subpopulations must utilize other biological markers. It is apparent from recent studies that cancer risk is the summation of the polymorphic nature of the bacterial population in the host, the host genotype, and environmental exposures, each affecting the level of long-term interactions between *H. pylori* and humans. Analytical tools now exist, however, including genome sequences (*H. pylori* and human), measurable phenotypes (CagA phosphorylation), and practical animal models, and may be used to discern the fundamental biological basis of *H. pylori*-associated neoplasia, which should have direct clinical applications. For example, persons with polymorphisms associated with high levels of IL-1β expression and who are colonized by *cag*[+] strains may be most likely to derive benefit from *H. pylori* eradication, as such treatment could result in a substantially reduced cancer risk. It is important to gain more insight into the pathogenesis of *H. pylori*-induced gastric adenocarcinoma, not only to develop more effective treatments for this common cancer but also because it might serve as a paradigm for the role of chronic inflammation in the genesis of other malignancies that arise within the gastrointestinal tract.

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