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Role of Innate Immunity in *Helicobacter pylori*-Induced Gastric Malignancy

Richard M. Peek Jr., **Chris Fiske**, and **Keith T. Wilson**
Divisions of Gastroenterology and Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, and Veterans Affairs Tennessee Valley Healthcare System, Nashville, Tennessee

Abstract

Helicobacter pylori colonizes the majority of persons worldwide, and the ensuing gastric inflammatory response is the strongest singular risk factor for peptic ulceration and gastric cancer. However, only a fraction of colonized individuals ever develop clinically significant outcomes. Disease risk is combinatorial and can be modified by bacterial factors, host responses, and/or specific interactions between host and microbe. Several *H. pylori* constituents that are required for colonization or virulence have been identified, and their ability to manipulate the host innate immune response will be the focus of this review. Identification of bacterial and host mediators that augment disease risk has profound ramifications for both biomedical researchers and clinicians as such findings will not only provide mechanistic insights into inflammatory carcinogenesis but may also serve to identify high-risk populations of *H. pylori*-infected individuals who can then be targeted for therapeutic intervention.

I. INTRODUCTION

Helicobacter pylori is a Gram-negative bacterial species that selectively colonizes gastric epithelium and is the most common bacterial infection worldwide (187,211). Virtually all persons infected by this organism develop gastritis, a signature feature of which is the capacity to persist for decades. Increasing evidence indicates that *H. pylori* is able to send and receive signals from cellular components within the gastric mucosa, allowing host and bacteria to participate in a dynamic equilibrium (35,210). However, there are biological costs to these long-term relationships.

Sustained interactions between *H. pylori* and humans significantly increase the risk for atrophic gastritis, intestinal metaplasia, and distal gastric adenocarcinoma, and colonization by *H. pylori* is the strongest identified risk factor for malignancies that arise within the stomach (56,195,207,210,280). Based on these data, the World Health Organization has classified *H. pylori* as a class I carcinogen for gastric cancer, and since virtually all infected persons have superficial gastritis, it is likely that the organism plays a causative role early in this progression (Fig. 1). Eradication of *H. pylori* significantly decreases the risk of developing gastric adenocarcinoma in infected individuals without premalignant lesions, providing additional evidence that *H. pylori* influences early stages in gastric carcinogenesis

Address for reprint requests and other correspondence: R. M. Peek, Mina C. Wallace Professor of Medicine and Cancer Biology, Vanderbilt University School of Medicine, 2215B Garland Ave., 1030C Medical Research Building IV, Nashville, TN 37232-2279 (richard.peek@vanderbilt.edu).

(298). However, only a fraction of colonized persons ever develop neoplasia, and disease risk involves specific and well-choreographed interactions between pathogen and host.

In this review, we discuss mechanisms through which *H. pylori* manipulates the innate immune system as a means to persist long-term within the gastric niche. The innate immune response in the gastrointestinal tract consists of many components, including pattern recognition receptors. These receptors recognize conserved microbial constituents termed pathogen- or microbe-associated molecular patterns such as flagellin, peptidoglycan, lipopoly-saccharide, and formylated peptides. Pattern recognition receptors are expressed on epithelial cells as well as neutrophils and include extracellular Toll-like receptors (described in detail later) and Nod-like receptors, which are housed intracellularly. In the gut, engagement of pattern recognition receptors triggers activation of conserved signaling cascades such as those mediated by nuclear factor κB (NF-κB), mitogen-activated protein kinases (MAPK), and caspase-dependent signaling pathways.

NF-κB constitutes a family of transcription factors sequestered in the cytoplasm, whose activation is tightly controlled by inhibitory IκB proteins (157,284). Multiple signals, including microbial contact, stimulate phosphorylation of IκB by IκB kinase β (IKKβ). This leads to proteasome-mediated degradation of phospho-IκB, thereby liberating NF-κB to enter the nucleus where it regulates transcription of a variety of genes, including immune response genes (157,176). MAPK are signal transduction net-works that target transcription factors such as AP-1 and mediate cytokine expression (93,129,240). MAPK cascades are organized in three-kinase tiers consisting of a MAPK, a MAPK kinase (MKK), and a MKK kinase (MKKK), and transmission of signals occurs by sequential phosphorylation and activation of components specific to a respective cascade. MAPK modules include ERK 1/2, p38, and JNK (93,129,240).

An understanding of how *H. pylori* manipulates the innate immune system will not only provide insights into the pathogenesis of gastric cancer but may also construct a paradigm for other cancers that arise from inflammatory foci within the gastrointestinal tract. Greater than 80% of hepatocellular carcinomas worldwide are attributable to chronic hepatitis B and hepatitis C infections, and cholangiocarcinoma of the biliary tract is strongly linked to chronic inflammation induced by certain parasites, such as *Opisthorchis* and *Clonorchis* (159). Chronic esophagitis, pancreatitis, and ulcerative colitis each confers a significantly increased risk for the development of adenocarcinoma within their respective anatomic sites. Thus a comprehensive understanding of how *H. pylori* dysregulates the innate immune response to initiate the progression to gastric cancer should facilitate understanding how chronic inflammation leads to malignant degeneration in other organ systems.

II. OBSTACLES TO COLONIZATION OF THE STOMACH THAT ARE OVERCOME BY *H. PYLORI*

One of the fundamental barriers to successful colonization of the stomach is peristalsis; consequently, *H. pylori* has evolved several mechanisms to elude this primary host defense including motility and adherence to gastric epithelium (Table 1). *H. pylori* possesses polar flagella, and its spiral shape permits efficient hydrodynamic movement within gastric mucous. Although the majority of *H. pylori* reside within the mucous gel layer, ~20% of the bacterial population binds to gastric epithelial cells (114). *H. pylori* expresses multiple paralogous outer membrane proteins (OMPs), several of which bind to defined receptors on gastric epithelial cells, and strains differ in both expression and binding properties of certain OMPs. BabA is an adhesin that binds the fucosylated Lewis^b receptor on gastric epithelial cells, while SabA binds sialyl Lewis^x receptors (128,162,252). Another differentially expressed OMP is OipA, which not only mediates cell binding but also triggers intracellular

signaling events that culminate in the release of proinflammatory cytokines and β-catenin activation (87,309).

Gastric acidity is another barrier that prevents colonization of the stomach. *H. pylori* grows optimally in vitro at neutral pH and fails to grow at pH levels below 4, indicating that this organism harbors mechanisms, such as production of urease, for surviving the low pH conditions inherent to the gastric niche. Indeed, initial infection with *H. pylori* leads to transient hypochlorhydria, perhaps in response to gastric inflammation (169,186), but the gastric pH decreases to within normal range within several months. Urease production by *H. pylori* not only combats the harsh acidic conditions of the stomach, but also alters the viscosity of gastric mucous, thereby optimizing motility.

The host immune system is another formidable obstacle that *H. pylori* must overcome to establish persistence and cause disease. Emerging data have indicated that *H. pylori* has multiple mechanisms to both evade and manipulate the immune response, which will be discussed in detail later in this review. Gram-negative bacteria, including *H. pylori*, possess lipopolysaccharide (LPS) as a component of the cell wall. LPS typically elicits a strong inflammatory response; however, the LPS of *H. pylori* is relatively anergic due to modifications of its lipid A component, having as little as 10^3 less endotoxin activity when compared with LPS from other Gram-negative bacteria (217). *H. pylori* LPS was recently shown to bind trefoil factor 1 (TFF1), a cysteine-rich protein found within gastric mucous, which likely promotes colonization (225). In contrast to flagella from other short-lived Gram-negative mucosal pathogens such as *Escherichia coli* or *Salmonella*, the flagella of *H. pylori* do not activate TLR5-mediated signaling, and inactivation of *flaA*, the major structural subunit of *H. pylori* flagellum, significantly reduced binding to TFF1 and altered production of *H. pylori* rough-type LPS (225). *H. pylori* has recently been shown to decrease production of specific heat shock proteins (HSPs) in vitro and within colonized gastric mucosa (25). Since HSPs can modulate both innate and adaptive immune responses, inhibition of HSP production may represent an additional mechanism of immune evasion that promotes long-term colonization.

Studies utilizing refined microscopy techniques have identified subpopulations of viable *H. pylori* within gastric epithelial cells and within the lamina propria (24,192,245), which may represent sanctuary sites to promote long-term persistence. *H. pylori* also has the capacity to usurp cholesterol from its host and incorporate this into its membrane (301), which could facilitate molecular mimicry. Thus *H. pylori* possesses numerous mechanisms that permit it to overcome obstacles presented by the stomach to successfully colonize its niche and subsequently impact gastric physiology.

III. MICROBIAL VIRULENCE CONSTITUENTS

Pathogenicity and virulence are terms that are difficult to distinguish but which have distinct meanings. A pathogen is traditionally viewed as a microbe that causes disease, although this is an overly simplistic definition. It is now clear that many microorganisms considered to be pathogens do not invariably cause overt disease. Therefore, a more refined concept of pathogenicity is that proposed by Casadevall and Pirofski (46) in which pathogenicity is defined as “the capacity of a microbe to cause damage in a host” and is due to microbial action against the host or the host response to the pathogen. Frequently, the term *virulence* has been equated with pathogenicity. A more accurate definition may be “the relative capacity of a microbe to cause damage in a host” (46) and is the definition that will be used for the purposes of this review. Two of the most well-studied virulence constituents of *H. pylori* are the *cag* pathogenicity island and *vacA*, which encodes the vacuolating cytotoxin.

A. The *H. pylori cag* Pathogenicity Island

H. pylori strains isolated from different individuals are extremely diverse (11,80,97,132,236,271), and one genetic determinant that augments cancer risk is the *cag* pathogenicity island, a 40-kb locus present in ~60% of strains in the United States (Fig. 2) (7,11,48,271). Although all *H. pylori* strains induce gastritis, cag^+ strains significantly augment the risk for severe gastritis, atrophic gastritis, and distal gastric cancer compared with strains that lack the *cag* island (Fig. 2) (36,58,64,65,[149],[206],[212],[213],[220],234,249,275,288).

1. The type IV secretion system—Elements contained within the *cag* island encode products that exert effects on the host by altering signaling pathways in gastric epithelial cells. Several *cag* genes encode proteins that bear homology to components of a type IV bacterial secretion system (TFSS) which functions to export microbial proteins, and the product of the terminal gene in the island (CagA) is translocated into host epithelial cells following bacterial attachment (Fig. 2). Two recent studies have now firmly implicated CagA as a bacterial oncoprotein by demonstrating that this molecule can attenuate apoptosis in vivo and in vitro and that transgenic expression of CagA in mice leads to the development of aberrant gastric epithelial proliferation and gastric carcinoma (180,201).

Following its injection into epithelial cells, CagA undergoes targeted tyrosine phosphorylation by Src and Abl kinases at motifs containing the amino acid sequence EPIYA, which are located within the 3′ terminus of CagA (Fig. 3) (23,27,196,243,244,255,268). The number and type of CagA EPIYA motifs can vary substantially (116), and motifs in strains harvested from persons residing in Western countries have been termed A, B, or C based on sequences flanking the EPIYA motif. In contrast, phosphorylation sites within CagA proteins from East Asian *H. pylori* strains lack the EPIYA-C motif and, instead, contain a different motif, which is termed D (116). *H. pylori* strains possessing more than three EPIYA motifs are more frequently associated with gastric atrophy, intestinal metaplasia, and gastric cancer (22,26,233,306), and in vitro, the number of EPIYA motifs is associated with the intensity of CagA phosphorylation, epithelial cellular elongation, and induction of proinflammatory cytokines (Fig. 3) (21,22,241). Within epithelial cells, phospho-CagA activates a eukaryotic phosphatase (SHP-2) as well as ERK, a member of the MAPK family, leading to morphological aberrations that mirror changes induced by growth factor stimulation (23,27,196,243,244,254,255).

Nonphosphorylated CagA also exerts effects within epithelial cells that contribute to pathogenesis. The cell adhesion protein E-cadherin, the hepatocyte growth factor receptor c-Met, the phospholipase phospholipase C (PLC)-γ, the adaptor protein Grb2, and the kinase Par1 have all been reported to interact with CagA in its nonphosphorylated form (55,181,188,235,314), and phosphorylation-independent CagA interactions induce proinflammatory and mitogenic responses as well as disruption of cell-to-cell junctions and loss of cell polarity. Nonphosphorylated CagA associates with the epithelial tight junction scaffolding protein ZO-1 and the transmembrane protein junctional adhesion molecule (JAM-A), leading to ectopic assembly of tight junction components at sites of bacterial attachment (16,29). Recently, the kinase Par1, a central regulator of cell polarity, was found to mediate this process, as CagA directly binds Par1, inhibits its kinase activity, and dysregulates mitotic spindle formation, thus promoting loss of cell polarity (154,235,281,314).

Integrin receptors are required for the successful injection of CagA (150), and an important role is played by CagL (Fig. 2), a T4SS pilus-localized protein, which links the T4SS to β_1-integrin on target cells (150). CagL also activates the host cell kinases focal adhesion kinase

(FAK) and Src to ensure that CagA is phosphorylated directly at its site of injection. β_1-Integrin is required for *H. pylori-induced* host cell motility and elongation (150), which is linked to phosphorylation of paxillin and the MAPK JNK (251). Binding of CagL to integrins also induces local membrane ruffling, suggesting that this interaction may exert a more global effect on membrane dynamics.

CagL contains an Arg-Gly-Asp (RGD) motif that mediates contact of the T4SS to $\alpha_5\beta_1$-integrin (150), but CagL can also bind in a RGD-independent manner to another integrin member ($\alpha_v\beta_5$) and fibronectin. Of interest, integrins are not found at the apical membrane but, instead, are present at the basolateral membrane of polarized cells, which may explain why *H. pylori* does not cause more extensive damage and may only inject effector proteins into target cells under certain circumstances and at specific sites. A working model based on these findings posits that microbial factors may disrupt intercellular epithelial junctions locally, thereby allowing only a limited number of bacteria to gain access to integrins and subsequently inject CagA (293).

2. Additional H. pylori cag island substrates that mediate pathogenesis—

Another consequence of *cag* island-mediated *H. pyvlori*-epithelial cell contact is induction of cytokine secretion, including the chemokine interleukin (IL)- 8 (67). IL-8 is typically secreted by gastrointestinal epithelial cells in response to pathogenic bacteria (75) and binds to the extracellular matrix, establishing a haptotactic gradient that directs inflammatory cell migration towards the epithelial cell surface (138,172–174). IL-8 is increased within *H. pylori*-infected mucosa (61,213,307) where it localizes to gastric epithelial cells (60), and levels of IL-8 are directly related to the severity of gastritis (213). Compared with *cag*$^-$ strains, *cag*$^+$ strains induce an enhanced IL-8 and inflammatory response in human tissue (61,213,307). Inactivation of *cagA*, *cagE*, *cagY*, and/or the entire *cag* locus also attenuates the development of inflammation in rodent models of *H. pylori*-induced injury, including Mongolian gerbils and hypergastrinemic INS-GAS mice (83,87,131,198).

The human IL-8 promoter contains binding sites for NF-κB, AP-1, and an interferon-stimulated responsive element (ISRE) (3,47,209,308). Contact between *H. pylori* and gastric epithelial cells in vitro results in brisk activation of NF-κB as well as ERK 1/2, p38, and JNK, which is followed by increased IL-8 expression (3,33,142,161,248,308), and *cag*$^+$ strains selectively activate these signaling cascades in gastric epithelial cells (66,143,144,179). Although several laboratories have shown that IL-8 induction is dependent on many *cag* genes, but not CagA (143,144,179,191,278), three recent studies reported that, in certain strains, CagA can induce IL-8 expression via NF-κB activation (40,145,151).

In addition to CagA, the *cag* secretion system can also deliver components of *H. pylori* peptidoglycan into host cells where they are recognized by Nod1, an intracytoplasmic pathogen-recognition molecule (39,285). Nod1 sensing of *H. pylori* peptidoglycan components activates NF-κB and regulates expression of the cytokine MIP-2 and β-defensin, and Nod1-deficient mice are more susceptible to infection by *H. pylori cag*$^+$ strains compared with wild-type mice (39,285). In support of these findings, recent data indicate that *H. pylori* proteins mediating the synthesis of peptidoglycan may also influence pathogenesis.

The *H. pylori* gene *slt* encodes a soluble lytic transglycosylase that is required for peptidoglycan turnover and release, thereby limiting the amount of peptidoglycan that is translocated into host epithelial cells (285). Inactivation of *slt* in *H. pylori* has now been shown to inhibit phosphatidylinositol 3-kinase signaling and *H. pylori*-induced cell migration (190), events that likely play a role in carcinogenesis. The protein encoded by the

H. pylori gene *HP0310* is homologous to polysaccharide deacetylase, an enzyme that catalyzes the hydrolysis of *N* -linked acetyl groups from *N*-acetylglucosamine residues or *O*-linked acetyl groups from *O*-acetylxylose residues. This enzyme also functions to deacetylate *N*-acetylglucosamine residues in *H. pylori* peptidoglycan (289), and Franco et al. (85) have recently demonstrated that loss of *HP0310* augments CagA translocation. Collectively, these results indicate that contact between cag^+ strains and gastric epithelial cells activates multiple signaling pathways that regulate innate immune cellular responses, which may heighten the risk for transformation, particularly over prolonged periods of colonization.

B. The *H. pylori* Vacuolating Cytotoxin

The *H. pylori* gene *vacA* encodes a secreted bacterial toxin (VacA) and represents another microbial locus linked with disease. All *H. pylori* strains contain a *vacA* gene, but there is marked variation in *vacA* sequences among strains. The regions of greatest sequence diversity are localized near the 5′ end of *vacA* (allele types s1a, s1b, s1c, or s2), the midregion of *vacA* (allele types m1 or m2), or the intermediate region (allele types i1 or i2) (Fig. 4*A*) (228). Variations in sequence are associated with variations in VacA functional activity in vitro, and *H. pylori* strains that possess s1/m1/i1 *vacA* alleles are associated with an increased risk of gastric cancer compared with *vacA* s2/m2/i2 strains (228).

1. Structural features of VacA—VacA undergoes cleavage during its transport through the bacterial membrane, and consistent with this, VacA contains a 33-amino acid signal sequence. Current models indicate that a 96-kDa VacA protein is secreted, which is then cleaved into an 88-kDa mature protein (p88) and a 10.5-kDa passenger domain (p10) (Fig. 4*B*). The mature, secreted p88 subunit can undergo further proteolytic cleavage to yield two fragments, p33 and p55 (34,57,270), which represent the two functional domains of VacA. Cell binding is mediated by the p55 fragment of VacA (227), but p33 and p55 can also exert multiple other effects.

VacA inserts into planar lipid bilayers to form anion-selective membrane channels (68,133,272). The p33 domain contains a hydrophobic sequence, which is involved in pore formation (171,286), whereas the p55 fragment contains one or more cell-binding domains (227). Since the p55 subunit contains the m1 and m2 alleles, delineation of protein sequences from unrelated *H. pylori* strains should allow identification of VacA structural features that are important for binding to host receptors (92).

2. Interactions between VacA and cellular receptors and vacuole formation—To determine how VacA contributes to *H. pylori*- induced disease, the effects of this protein on human cells have been investigated in vitro and in vivo. Similar to other toxins, VacA interacts with the plasma membrane of target cells as a first step during intoxication (57). VacA targets multiple epithelial cell-surface components to initiate this process, including RPTPβ (89,303), RPTPα (304), fibronectin (112), the epidermal growth factor (EGF) receptor (246), heparin sulfate (282), various lipids (68,183,185) and sphingomyelin (105), as well as CD18 (integrin β2) on T cells (247).

One of the proteins to which VacA binds is the receptor-type protein tyrosine phosphatase, RPTPβ (89). Protein tyrosine phosphatases constitute a diverse family of cytoplasmic and transmembrane receptor-like enzymes that regulate proliferation, differentiation, and adhesion. Since RPTPβ regulates cellular phenotypes that may contribute to mucosal damage, the role of VacA-RPTPβ interactions in gastric injury has been studied in depth using complementary in vivo and ex vivo genetic models of RPTPβ deficiency. The ability of VacA to bind to RPTPβ in gastric tissue was determined by passing gastric mucosal

homogenates from wild-type and RPTPβ-deficient mice across in silico VacA-laden surfaces (89). The binding capacity of RPTPβ$^{-/-}$.extracts was 30% less than wild-type, differences that resolved in the presence of antagonistic RPTPβ antibodies (89). Purified VacA has also been delivered via gavage to wild-type and RPTPβ- deficient mice; VacA induced gastric injury only in RPTPβ$^{+/+}$ wild-type mice, and the majority of wild-type mice challenged with VacA developed severe gastric hemorrhage and ulcers (89).

VacA not only interacts with gastric epithelial cells but also with immune cells including macrophages, B cells, and T cells. VacA enters activated primary human T lymphocytes by binding to the CD18 receptor and exploiting the recycling of LFA-1 (247). LFA-1-deficient Jurkat T cells are resistant to vacuolation, and genetic complementation restores sensitivity to VacA. Interestingly, VacA targets human, but not murine, CD18 for cell entry, consistent with the species-specific adaptation of *H. pylori* (247).

A current model for how VacA induces vacuole formation is that VacA binds to the plasma membrane of target cells, is internalized, and forms anion-selective channels in endosomal membranes and then vacuoles arise due to swelling of endosomal compartments. VacAinduced vacuoles are hybrid compartments of late endosomal origin that contain lysosomal markers (184). Vacuole production depends on the presence and activity of a number of factors such as the V-ATPase (203), as well as three GTPases: Rab7 (204), Rac1 (124), and dynamin (258). The protein kinase PIKfyve is also necessary for vacuole formation, and microinjection of its substrate phosphatidylinositol 3,5-bisphosphate (PIP_2) is sufficient to induce vacuoles (127). Vacuolating activity also depends on VacA oligomerization (295,311) and requires the hydrophobic NH_2 terminus containing three tandem GXXXG motifs, which are transmembrane dimerization sequences (171). These findings indicate that channel formation is a prerequisite for vacuolation, which is supported by the finding that pharmacological anion-channel inhibitors block vacuole formation (261).

3. Effects of VacA on cellular responses with carcinogenic potential—One of the defining characteristics of functional epithelia is the development of transepithelial electrical resistance (TER), and in polarized monolayers, VacA has been shown to decrease TER (205). This effect was observed in several different polarized models including MDCK, T84, and EPH4 cells and was independent of its vacuolating activity (216). Although the mechanism by which VacA alters paracellular permeability is not yet completely understood, VacA increases the transepithelial flux of certain molecules such as urea (273). However, other studies have indicated that disruption of cell-cell junctions in polarized epithelia by *H. pylori* is not dependent on VacA per se, but also requires injected CagA which targets both tight and adherens junctions. More complexity arises from another recent study showing that *H. pylori* induced a progressive loss of barrier function in MKN28 gastric epithelial cells and INS-GAS mice, which was attenuated by inactivation of the *ureB* gene, but not *vacA* or *cag* island genes (300). Thus epithelial barrier disruption induced by *H. pylori* appears to require multiple bacterial factors and signaling pathways (293).

Infection with *H. pylori* has been associated with both increased and reduced levels of apoptosis in the gastric epithelium (34,210). In vitro, *H. pylori* reproducibly stimulates apoptosis in infected gastric epithelial cells, and early studies indicated that purified VacA induced mitochondrial damage as reflected by dramatic decreases in cellular ATP levels (146). In transfected HEp-2 cells, the VacA p33 fragment localizes specifically to mitochondria, whereas p55 is cytosolic (91). Transient expression of p33-GFP or VacA-GFP in HeLa cells induced the release of cytochrome *c* from mitochondria leading to activation of caspase-3. These findings have been supported by studies using wild-type and *vacA* mutant *H. pylori* strains (59,148). Interestingly, the s1/m1 type of VacA induces high levels of apoptosis compared with a s2/m1 toxin or VacA mutants lacking the hydrophobic NH_2-

terminal region. These results indicate that VacA induces gastric epithelial cell apoptosis and suggest that differences in levels of apoptosis among *H. pylori*-infected persons may result from strain-dependent variations in VacA structure. However, *H. pylori* has recently been shown to inhibit gastric epithelial cell apoptosis in Mongolian gerbils (180), which was associated with epithelial hyperplasia and persistent bacterial colonization of the stomach. In that study, suppression of apoptosis was mediated by CagA, which stimulated the pro-survival MAPK ERK1/2 and the anti-apoptotic protein MCL1 within gastric pits. Thus CagA may counteract the effects of VacA and activate cell survival and anti-apoptotic pathways to overcome self-renewal of the gastric epithelium and help sustain *H. pylori* infection (180).

VacA can also exert detrimental effects on the host immune response. Purified VacA can inhibit processing and presentation of antigenic peptides to human $CD4^+$ T cells (183), and in professional phagocytes such as human THP-1 and mouse RAW 264.7 macrophage cell lines, wild-type *H. pylori* displays enhanced survival compared with *vacA* deletion mutants (316). However, increased survival of *vacA* mutants was not seen in two other studies when freshly isolated human monocytes were used (224,230), and these differences may be due to different *vacA* alleles, infection doses, and/or cell types.

Infection or coincubation of purified VacA with T lymphocytes yields multiple effects. VacA specifically blocks antigen-dependent proliferation of T cells by interfering with IL-2-mediated signaling (37,95), and, as discussed above, *H. pylori* coopts CD18 β_2-integrin as a VacA receptor on human T lymphocytes (247). After cell entry, VacA inhibits Ca^{2+} mobilization and downregulation of the activity of the Ca^{2+}-dependent phosphatase calcineurin. This, in turn, inhibits activation of the transcription factor nuclear factor of activated T cells (NFAT), and NFAT target genes such as IL-2 and the high-affinity IL-2 receptor (IL-2Rα) are not expressed. VacA, however, exerts a different effect on primary human $CD4^+$ T cells (257) via suppressing IL-2-induced cell cycle progression and proliferation of primary T cells in an NFAT-independent manner. Thus VacA may inhibit the clonal expansion of T cells that has been activated by bacterial antigens, thereby allowing *H. pylori* to evade the adaptive immune response.

IV. TRANSLATIONAL MODELS OF *H. PYLORI*-INDUCED INJURY

Animal models have provided valuable insights into critical mediators that are involved in *H. pylori*-induced gastric carcinogenesis in vivo. Rodents and primates represent the primary models that have been utilized and are complementary systems. Mice are inbred with defined genotypes, and transgenic lines allow a more detailed analysis of host susceptibility to *H. pylori* virulence determinants. Mongolian gerbils are outbred, which increases the variability in response to any stimulus; however, gerbils can develop cancer when colonized with certain strains *of H. pylori*. Primates are the most closely related model to the human host, but experimental manipulations in this system cannot be conducted on the same scale as rodents; therefore, large studies are impractical due to costs.

Infection of mice with *H. pylori* cag^+ strains frequently leads to deletions within the *cag* island (218,253). In contrast, *H. pylori* reproducibly induces gastric inflammation in gerbils, and *H. pylori* mutant strains colonize this model well (131,215). Compared with gerbils infected with wild-type *H. pylori*, gerbils colonized with *cag* island mutant strains develop significantly less severe gastritis (131,198). Loss of *cagA* or *cagY has* also been reported to result in an inflammatory response that is primarily restricted to the gastric antrum, and which does not significantly involve the acid-secreting corpus (229). Thus a functional *cag* secretion system is required to induce corpus-predominant gastritis, a precursor lesion in the progression to gastric adenocarcinoma (229). Long-term *H. pylori* infection of gerbils can

also lead to gastric adenocarcinoma (86,121,292), and our laboratory has demonstrated that development of gastric cancer in this model is dependent on CagA (87).

Wild-type mice are not as susceptible to *H. pylori*-induced injury as gerbils; therefore, *Helicobacter felis* has been used to induce gastric injury in mice since the degree of gastric damage is usually more severe in mice infected with *H. felis* compared with *H. pylori*. Gastric adenocarcinoma can also develop following long-term infection of wild-type C57/Bl6 mice with *H. felis* (44). However, many *H. pylori* virulence components, such as the *cag* pathogenicity island and *vacA*, are not present within the *H. felis* genome.

Transgenic mice have now been generated that are more susceptible to gastric cancer than wild-type mice, which has provided insights into host factors that mediate human gastric carcinogenesis. For example, mutation of the IL-6 family coreceptor gp130 leads to altered SHP-2 signaling and constitutive activation of STAT3, which culminates in the development of intestinal-type gastric adenocarcinoma in genetically engineered mice (137,269). Transgenic mice that overexpress gastrin (INS-GAS mice) spontaneously develop gastric cancer, but this requires the virtual lifetime of the animal (290). Concomitant infection with the mouse-adapted *H. pylori* strain SS1 or the gerbil-adapted *H. pylori* strain 7.13 accelerates this process (83,84), suggesting that persistently elevated gastrin levels synergize with *H. pylori* to augment the progression to gastric cancer.

Primates have also been used to examine the role of *H. pylori* genes on induction of disease. In *H. pylori*-infected Rhesus monkeys, *CagA* is expressed at high levels during the entire time course of infection. In contrast, some *Cag* genes (*e.g., CagY*) were more highly expressed 1 wk postinfection compared with later time points, while expression of others (*e.g., CagC*) increased between 2 and 3 mo and then fell by 4–6 mo postchallenge (38). Collectively, these data indicate an important role for CagA and other products of the *Cag* pathogenicity island in the development of *H. pylori*-induced disease, particularly gastric cancer.

In contrast to the *Cag* island, multiple studies have been unable to detect a significant difference in levels of injury induced by wild-type versus *vacA* mutant *H. pylori* strains in animal models (73,104,297), although one study demonstrated that a *vacA* mutant was less efficient than wild-type *H. pylori* in colonization of mice (237). However, oral delivery of purified VacA induced gastric inflammation, hemorrhage, and ulcers in wild-type mice, which may contribute to *H. pylori*-induced ulcerogenesis in humans (89).

Several *H. pylori* adhesins have also been studied in animal models. Solnick et al. (252) reported that the gene encoding BabA (*babA2*) could be replaced with a highly related gene *babB* via recombination in *H. pylori*-infected Rhesus monkeys. SabA is an *H. pylori* adhesin that binds the sialylated glycan sialyl-Le^X (162), and experimental infection of primates with a sialyl-Le^X-binding *H. pylori* strain revealed that *H. pylori* induces increased expression of sialyl-Le^X in the gastric epithelium (162). Thus studies utilizing animal models for the study of *H. pylori*-induced diseases will continue to provide important information regarding the mechanisms of gastric carcinogenesis in vivo.

V. *H. PYLORI* INTERACTIONS WITH A CRITICAL COMPONENT OF THE INNATE IMMUNE SYSTEM: GASTRIC EPITHELIAL CELLS

Delineation of mechanisms that regulate complex biological processes, such as microbially induced cancer, requires the use of in vitro models in which organisms encounter host factors similar to those present in human infection. One of the initial components of the innate immune response to be encountered by *H. pylori* in the stomach is the gastric

epithelial cell. Contact between *H. pylori* and epithelial cells in vitro dysregulates signaling pathways that influence inflammation and oncogenesis, and this is mirrored by *H. pylori-epithelial* interactions that occur within infected human and rodent gastric tissue. In transgenic mice that overexpress Lewis^b, *H. pylori* adhere directly to gastric epithelial cells (79,106). Genetic ablation of parietal cells in Lewis^b-expressing transgenic mice permits the gastric epithelial progenitor (GEP) cell population to expand, which is accompanied by an expansion of *H. pylori* colonization and inflammation within the glandular epithelium (259,260). *H. pylori* has the capacity to directly interact with GEP cells (199), and delineation of the GEP transcriptome has identified several pathways that are overrepresented in this lineage and which are of particular biological importance for carcinogenesis, including Wnt/β-catenin (200). On the basis of these findings, a myriad of studies have focused on aberrant epithelial responses induced by pathogenic *H. pylori*, which provides a foundation for understanding the critical importance of the host innate immune response in gastric carcinogenesis (125,126).

H. pylori is a human pathogen; therefore, most investigations focused on *H. pylori-epithelial* interactions have utilized human gastric epithelial cells. AGS human gastric epithelial cells (ATCC CRL-1739) are one of the most frequently used models for in vitro *H. pylori* studies and were derived from a patient with gastric adenocarcinoma. AGS cells possess wild-type p53 and APC (136), and several groups have demonstrated that the interaction between AGS cells and *H. pylori* is a useful in vitro model for specific aspects of pathogenesis in vivo, such as production of innate immune cytokines (66,131,214). AGS cells lack E-cadherin, however, which diminishes their usefulness as an appropriate model for studies focused on epithelial permeability.

MKN28 cells (JCRB0253) represent another commonly used human gastric epithelial cell model and were derived from a patient with a moderately differentiated tubular gastric adenocarcinoma. In addition to mirroring the ability of AGS cells to produce cytokines in response to *H. pylori*, MKN28 cells have recently been shown to form functional tight junctions as determined by transepithelial resistance (300), permitting studies that focus on *H. pylori* dysregulation of apical-junctional complexes. However, this cell line contains mutant p53, which should be considered when selecting this particular model. Other human cell models that have been used to study the effects of *H. pylori* on gastric epithelial cells include KATO III cells and AZ-521 cells.

One limitation of currently used in vitro models *of H. pylori-gastric* epithelial interactions is a dependence on transformed cells. To circumvent this, transgenic mice have been generated (Immortomice) that harbor a temperature-sensitive mutation of the simian virus 40 large-T antigen under the control of an interferon (IFN)-γ-inducible promoter (134,135,294). At normal mouse body temperature, the gene product is inactive. Functional T-antigen expression and immortalization can be induced by culturing isolated cells in vitro with IFN-γ at a temperature (33°C) permissive for function of the thermolabile mutation. When the gene product is inactivated at a nonpermissive temperature (37°C), cells acquire a finite lifespan, similar to primary cells (134,135,294). These cells have been used to demonstrate that *H. pylori* activates β-catenin and transactivates the EGF receptor (EGFR) (86,190,310).

Interactions between gastric epithelial and stromal cells are also important determinants of host innate immune responses within the stomach (170). Therefore, ex vivo murine gastric gland culture models have been developed to more fully recapitulate interactions that occur between *H. pylori* and gastric epithelial cells in vivo. Ogden et al. (197) have recently shown that infection of ex vivo gastric glands with *H. pylori* induces mislocalization of p120, a member of the catenin family, from the epithelial cell membrane to the nucleus where it colocalizes with the transcriptional repressor Kaiso and induces expression of the tumor-

associated matrix metalloproteinase (MMP)-7 (197). Thus the effects of *H. pylori* in vitro can be recapitulated ex vivo, which allows extension of mechanistic observations in cell culture into a physiologically relevant system of cellular organization.

VI. ROLE OF INNATE IMMUNE RECEPTORS IN GASTROINTESTINAL IMMUNITY

Toll-like receptors (TLRs) are constituents of a larger family, termed pattern-recognition receptors (PRRs), which play an essential role in initiating an innate immune response against invading microbes with subsequent activation of an adaptive immune response (266). Toll receptor was originally identified in *Drosophila* as an essential receptor for embryonic development (111). Toll-mutant flies were found to be profoundly susceptible to fungal infections, and mammalian homologs of Toll (TLRs) that participated in innate immune recognition of pathogens were later discovered (175). TLRs are an effective early warning system based on their capacity to recognize distinct highly conserved pathogen-associated molecular patterns (PAMPs) that are unique to microorganisms but which are absent from host cells. Recognition of PAMPs by TLRs leads to the activation of intracellular signaling pathways that culminate in the induction of various genes involved in host defense including those encoding inflammatory cytokines, chemokines, antigenpresenting molecules, and costimulatory molecules.

TLRs share a common structure consisting of an extracellular domain comprised of leucine-rich repeats and an intracytoplasmic domain that shows high similarity to the IL-1 receptor family, termed the Toll/IL-1 receptor (TIR) domain (Fig. 5). To date, 13 TLRs have been identified, and these can be differentiated by ligand specificity. Certain TLRs, (e.g., TLR3, TLR5, and TLR9) recognize only one type of PAMP, while others, such as TLR2, recognize a variety of molecules including bacterial lipoproteins, lipoteichoic acid, and peptidoglycan. TLR3 binds double-stranded RNA found in many types of viruses, TLR5 binds flagellin, and TLR9 recognizes repetitive sequences of unmethylated nucleic acids, or CpG repeats, which are present in high quantities in bacterial DNA. TLR4 recognizes LPS, an interaction that involves the transfer of LPS to CD14 via LPS-binding protein (LBP). CD14 then presents LPS to TLR4, in close association with a small secreted protein, MD-2, which is present on the cell surface (130).

In addition to being important effectors on immune cells, TLRs have been identified on gastrointestinal epithelial cells (45). Because of the continuous presence of microorganisms in the gut, the balance between TLR expression and activation is tightly regulated within this niche. It is crucial that TLRs are not triggered incessantly in response to PAMPs of commensal bacteria, but they still must harbor the capacity to activate appropriate downstream signaling pathways in the presence of potential pathogens. This can be accomplished by downregulation of specific TLRs, such as TLR2, TLR3, and TLR4, on the surfaces of human colonic and intestinal epithelial cells (1,2,90). In vitro studies have demonstrated that, upon stimulation with LPS or peptidoglycan, as occurs with constant exposure to commensal bacteria, TLR2 and TLR4 are redistributed from the apical surface to intracytoplasmic compartments adjacent to the basolateral membrane (45) or, in the case of TLR4, to the Golgi apparatus (122). However, despite this cell surface downregulation, TLR4 localized within the Golgi apparatus still retains full capacity to initiate intracellular signaling cascades in response to internalized LPS (123).

A. TLRs and *H. pylori* Infection

The primary barrier in the gastrointestinal tract against pathogens is the epithelial cell monolayer, and infection with *H. pylori* begins with adherence to gastric epithelial cells.

While TLRs have been detected on the surface of gastrointestinal epithelial cells, the precise role that TLRs play in the immune response to *H. pylori* remains controversial despite extensive studies in this area.

1. TLR4—Many studies focused on the innate immune response to *H. pylori* have centered on TLR4, which recognizes LPS. While TLR4 clearly mediates the initial recognition of pathogens by macrophages, there is no consensus regarding the role that TLR4 plays in the recognition of *H. pylori*. Infection of a gastric epithelial cell line with *H. pylori* increased expression of TLR4 and MD-2, and stimulation with *H. pylori* LPS led to activation of NF-κB and the IL-8 promoter in cells that expressed both receptors (130). However, findings from other investigators have suggested that recognition of *H. pylori* is TLR4 independent (28,256). Bäckhed et al. (28) reported that, although gastric mucosa specimens expressed TLR4, the inflammatory cytokine response to *H. pylori* was not directed towards LPS and was, therefore, TLR4 independent (28). Furthermore, while *H. pylori* can upregulate expression of TLR4 and use this receptor to adhere to gastric epithelial cells, a monoclonal antibody to TLR4 failed to inhibit induction of IL-8 secretion (256). One hypothesis for why TLR4 may not be involved in immune recognition of this pathogen is that *H. pylori* LPS is an ineffective activator of the immune response compared with LPS from other Gram-negative bacteria, due to modifications in the LPS lipid A core (217). Human macrophages differ in their ability to respond to LPS depending on the degree to which LPS is acylated, and a stronger immune response is evoked by hypoacylated LPS (109). Since *H. pylori* LPS is hypoacylated, it may not activate TLR4 effectively, thereby enabling the bacterium to survive long-term.

In addition to in vivo studies, genetic studies have been performed to define the role of TLR4 in the innate immune response to *H. pylori*. Arbour et al. (20) described a functional polymorphism at position +896 in exon 4 of the *TLR4* gene that replaces a conserved aspartic acid residue with glycine at amino acid 299 (Asp299Gly) and which alters the extracellular domain of TLR4 (20). This single nucleotide polymorphism (SNP) cosegregated with a missense mutation that replaced a nonconserved threonine with an isoleucine at amino acid 399 (Thr399Ile). The cosegregating mutant was found to be present more commonly in healthy individuals that were unresponsive to aerosol challenge with LPS (i.e., demonstrated less airway reactivity), which provided the first direct evidence that a sequence polymorphism in *TLR4* was associated with an endotoxin hyporesponsive phenotype in humans. Recent work has suggested that defective signaling via TLR4 may also result in an exaggerated immune response after the initial failure of innate immunity to control infection (117). Since such a response may allow *H. pylori* to induce chronic inflammation that leads to gastric cancer, Hold et al. (120) undertook separate case-control studies of individuals with precancerous phenotypes such as gastric atrophy, as well as individuals with gastric cancer, and evaluated these populations for the presence of the Asp299Gly SNP in TLR4. *TLR4* +896G carriers had a significantly increased risk for hypochlorhydria and gastric atrophy compared with controls (OR 11.0, 95% CI 2.5–48). The *TLR4* variant was also significantly associated with gastric cancer when compared with the control population (OR 2.4, 95% CI 1.6–3.4). However, several studies in different populations failed to identify an association between TLR4 Asp299Gly or Thr399Ile polymorphisms and gastric cancer or precancerous lesions (94,119,141,262,279), potentially due to noninformative haplotype distributions, although one group did identify an association between a novel polymorphism, *TLR4*+3725 G/C, and severe gastric atrophy in a Japanese population (118).

2. TLR2—TLR2 recognizes diverse PAMPs such as bacterial lipoproteins, lipoteichoic acid, and peptidoglycan and is often complexed as a heterodimer with TLR1 or TLR6. TLR2 is expressed on the surfaces of intestinal and gastric epithelial cells (45,122,250), and

several studies have suggested that TLR2 plays a role in recognition of *H. pylori* and subsequent induction of intracellular signaling cascades that activate inflammation. HEK293 cells transfected with TLR2 respond to different strains *of H. pylori* by activating NF-κB, whereas cells transfected with TLR4 fail to activate NF-κB (250). This same study found that gastric epithelial cells transfected with a dominant-negative TLR2 mutant (but not TLR4) were attenuated in their response to *H. pylori*, and purified *H. pylori* LPS is a predominantly TLR2, versus a TLR4, agonist. However, Mandell et al. (165) determined that while *H. pylori- derived* LPS stimulated TLR4, only TLR2 responded to intact bacteria.

Focusing on acute inflammation, another study found that human neutrophils infected with *H. pylori* upregulated expression of both TLR2 and TLR4, and the production of IL-8 and IL-10 was abrogated by the use of neutralizing anti-TLR2 and anti-TLR4 antibodies (12). However, these findings are not uniformly consistent across different laboratories. Viala et al. (285) reported that *H. pylori* infection of HEK293 cells, which do not express TLR2, resulted in a proinflammatory response, which likely was triggered by the intracellular host defense molecule Nod1. These results were later confirmed as stimulation of HEK293 cells with *H. pylori* resulted in activation of NF-κB, indicating that TLR2 may not play a major role in the immune response to *H. pylori* (160).

In addition to recognition of LPS, TLR2 can sense other *H. pylori* antigens leading to subsequent induction of immunomodulatory pathways. HSP60, a potent immunogenic antigen of *H. pylori*, can induce inflammatory cytokine production from gastric epithelial cells and monocytes via TLR2 (267,315). *H. pylori* neutrophilactivating protein (HP-NAP) is a virulence factor that stimulates production of oxygen radicals from neutrophils and facilitates adhesion of neutrophils to endothelial cells (78). HP-NAP is a TLR2 agonist that can elicit Th1 inflammatory cytokine production by neutrophils and monocytes (13).

Cyclooxygenase-2 (COX-2) expression is induced by *H. pylori*, and this has been implicated in the development and progression of gastric cancer (265,283). Chang and co-workers (49,50) investigated the role of TLRs in COX-2 expression induced by *H. pylori* infection in two independent studies. In one report, COX-2 expression occurred via TLR2- and TLR9-dependent pathways, which required NF-κB activation (50). In a subsequent study, TLR2 and TLR9 were found to play pivotal roles in COX-2 overexpression, which led to gastric cancer cell invasion and angiogenesis (49).

The role that *TLR2* polymorphisms play in the immune response to *H. pylori* has also been investigated. Two studies examined the −196 to −174del polymorphism of *TLR2*, which has previously been reported to decrease transactivation of TLR2-responsive promoters (194,263,264). This *TLR2* deletion was present in a higher proportion of Japanese patients with gastric cancer compared with persons who had ulcer disease, gastritis, or normal mucosa at endoscopy (263). In a subsequent study, this same deletion was found to be increased in patients with intestinal metaplasia, a precursor lesion in the cascade to gastric carcinogenesis (Fig. 1).

3. TLR5—TLR5 binds flagellin; therefore, it seems inherently obvious that TLR5 would play a pivotal role in the recognition of *H. pylori*, a flagellated bacterium. TLR5 is present on intestinal epithelial cells (45,122,250) and gastric epithelium (152). However, similar to data for TLR4 and TLR2, conflicting reports exist regarding the involvement of TLR5 in the immune response to *H. pylori*. HEK293 cells that are transfected with TLR5 (and TLR2 as noted above) respond to infection with *H. pylori* via activation of NF-κB (250). Furthermore, cotransfection of a gastric epithelial cell line, MKN45, with dominant-negative mutants of TLR2 or TLR5 resulted in inhibition of NF-κB activation in response to *H. pylori*, and purified *H. pylori* was found to activate NF-κB in HEK293 and MKN45 cells.

HEK293 cells have also been shown to produce IL-8 in response to *H. pylori*, and transfection of these cells with TLR5 or TLR2 augments this response via activation of p38, a member of the MAPK family (274).

However, data from other studies are not consistent with these findings and suggest that TLR5 does not play a major role in the immune response to *H. pylori. H. pylori* flagellins have been shown to harbor low activity in stimulating gastric epithelial cells via TLR5 (152), and while TLR5 expression on the surface of gastric epithelial cell lines can be modulated *by H. pylori*, flagellins do not play a role in regulation of these events. *H. pylori* flagellin has no effect on IL-8 secretion by gastric epithelial cells compared with flagellin from *Salmonella typhimurium* (96). Andersen-Nissen et al. (17) provided molecular details on mechanisms that may govern this nonresponsive phenotype by demonstrating that *H. pylori* flagellin contains specific amino acid substitutions within the TLR5 recognition site that render it nonstimulatory. An aflagellated clone *of H. pylori* has been shown to induce a proinflammatory response in HEK293 and gastric epithelial cells, indicating that TLR5 may not be important in epithelial cell recognition of *H. pylori* (285). Collectively, these findings strongly suggest that TLR-mediated responses to *H. pylori* must be interpreted with caution, as results may differ in a cell-context or *H. pylori* strain-context manner.

VII. ROLE OF IMMUNE CELLS IN *H. PYLORI*-INDUCED CARCINOGENESIS

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. Colonization of *H. pylori* can be abrogated by immunization with bacterial components such as urease (202), indicating activation of the adaptive response, but urease is also a major inducer of innate responses in monocytes and macrophages, stimulating cytokine and nitric oxide generation (101,163,164). Thus distinguishing 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 the interaction of activated T cells, illustrates the complexity of the host immune response.

A common theme in many diseases is the persistence of viral, bacterial, or parasitic infection with the resulting tissue damage deriving largely from the inflammatory host response that can predispose to neoplastic transformation. In addition to *H. pylori*, prototypical examples of microbial colonization of mucosal surfaces or epithelial cells leading to such consequences include human papilloma virus and cervical cancer, hepatitis C and B viruses leading to hepatocellular carcinoma, Epstein-Barr virus and nasopharyngeal carcinoma, and the parasitic helminths *Opisthorchis* and *Clonorchis* and cholangiocarcinoma as well as *Schistosoma haematobium* and bladder carcinoma (113).

H. pylori induces both humoral and cellular immune responses. Local and systemic antibody responses have been demonstrated that include IgA, IgM, and IgG istotypes (63), and early studies in mouse models demonstrated that immunization with *H. pylori* antigens could produce protective immunity (168). *H. pylori* causes an inflammatory reaction with both polymorphonuclear and mononuclear cells (102), and gastric mucosa of infected patients contains increased levels of proinflammatory cytokines such as IL-β, tumor necrosis factor (TNF)-α, IL-8, and IL-6 (61,62).

Although *H. pylori* proteins had been demonstrated in the lamina propria of the stomach, this organism has generally been considered to be 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 (15) and in vivo in the stomachs of humans and monkeys (245), as well as in mice with atrophic gastritis (199). The bacteria have also been shown to be bound to erythrocytes within the microvessels of the

lamina propria (24). Transmission electron microscopy and immunogold detection demonstrated that *H. pylori* are in direct contact with immune cells of the lamina propria in the majority of cases of gastritis and gastric cancer (192). These studies provide additional relevance for numerous important studies of the host immune cell responses to *H. pylori*, many of which have been accomplished through the use of reductionist in vitro and ex vivo approaches.

A. Neutrophils

Phagocytosis is a critical component of the innate immune response to invading pathogens. Engulfment of microbes by neutrophils leads to killing by oxygen-dependent and/or oxygen-independent mechanisms. A characteristic feature of *H. pylori* infection is migration of neutrophils or polymorphonuclear leukocytes (PMNs) into the gastric mucosa with subsequent inflammation. Although *H. pylori* plays a key role in promoting the migration of PMNs to the mucosa, it is able to survive in this hostile environment by manipulating phagocytosis and the subsequent oxidative burst.

The HP-NAP is a secreted virulence factor that promotes neutrophil recruitment and induces production of reactive oxygen radicals (219,239). HP-NAP acts via engagement of TLR2 to activate NF-κB with subsequent upregulation of IL-12, IL-23, and TNF-α (13). HP-NAP also modulates the adaptive immune response by influencing the production of T helper (Th)1 cells over Th2 cells, resulting in increased production of IFN-γ, TNF-α, and increased cytolytic activity of Th cells

To survive long-term within foci of gastritis, however, *H. pylori* has developed several mechanisms to avoid PMN-dependent killing. *H. pylori* avoids opsonization due to the low pH and mucins that are present within the local gastric environment, which prevent antibody binding to the bacterial surface (32). Urease produced by *H. pylori* prevents deposition of C3 (232). In addition, infection with *H. pylori* results in upregulation of decay-accelerating factor and CD59, both of which inhibit complement-mediated opsonization (238).

H. pylori also impedes phagocytosis by several mechanisms. Disruption of genes within the *cag* pathogenicity island enhances engulfment, indicating an important function of the type IV secretion system in preventing phagocytosis (224). Delayed phagocytosis is also mediated by a novel host signaling cascade driven by atypical protein kinase C (PKC)-ζ (8), which is distinct from conventional phagocytosis of pathogens that is dependent on PKC-α and PKC-δ. Finally, the unique lipid composition of the *H. pylori* outer membrane influences uptake of the bacterium into phagocytes; specifically, glucosylation of cholesterol in the outer membrane increases the ability of *H. pylori* to evade phagocytosis, whereas an excess of cholesterol leads to enhanced phagocytosis (301).

Following phagocytosis, *H. pylori* can also survive within PMNs by disrupting the NADPH oxidase system that synthesizes reactive oxygen species (ROS). While PMNs containing *H. pylori* produce a substantial amount of ROS, these species do not accumulate inside the phagosomes, and NADPH oxidase assembly in the phagosome is inefficient (9). Rather, the NADPH oxidase system assembles on the PMN cell surface and releases ROS into the extracellular space, resulting in increased local inflammation.

Thus the relationship between *H. pylori* with PMNs is complex. The bacterium uses virulence factors to recruit PMNs to the gastric mucosa, which favors local tissue damage and releases essential nutrients. However, *H. pylori* must evade this induced response so that it is not eliminated. To promote its survival, the bacterium modulates phagocytosis through production of bacterial constituents and dysregulation of host signaling pathways and diverts ROS formation away from the phagosome and into the extracellular space.

B. Macrophages

Macrophages are essential 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 (107,177,178) that stimulate Th1 cells, resulting in production of cytokines such as IFN-γ. 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 (13). IL-12 production in the gastric mucosa is linked to the development of peptic ulcers in infection with *H. pylori cag*$^{+}$ strains, most likely due to stimulation of Th1 responses (115). Macrophages are also involved in amplification of the inflammatory response by production of cytokines such as IL-1, TNF-α, and IL-6 (98,110,164), and IL-6 activation has been linked to activation of TLR4, MAPK, and NF-κB signaling events (208).

Macrophages also function as effector cells in host defense. One such pathway involves generation of nitric oxide (NO) derived from the enzyme inducible NO synthase (iNOS, NOS2), which has been shown to be upregulated by *H. pylori* in macrophages in vitro (43,99,100,296) and in vivo (88,167). Events involved in the host iNOS response to *H. pylori* are illustrated in Figure 6. Coculture studies demonstrate that *H. pylori* can be killed by macrophages even when physically separated from these effector cells by a filter support and that this antimicrobial defense is NO dependent (43,100). The arginase enzyme possessed by *H. pylori* and encoded by the gene *rocF* can compete sufficiently with macrophages for the iNOS substrate L-arginine (L-Arg) such that host NO production is impaired, leading to enhanced survival of the bacterium (100). Bacterial arginase generates urea from L-Arg, which is then utilized by urease to synthesize ammonia that is required to neutralize gastric acid. However, attenuation of macrophage NO generation additionally benefits *H. pylori* by enhancing immune evasion. Another example of the ability of *H. pylori* to escape the macrophage response is via glucosylation of cholesterol, and mutant strains that cannot process cholesterol have increased susceptibility to phagocytosis by macrophages and cannot colonize the mouse stomach (222).

While induction of iNOS in macrophages is termed classical activation or the M1 type, an alternative, M2 pathway involving the metabolism of L-Arg by arginase is also involved (Fig. 7). Exposure of macrophages to *H. pylori* products results in upregulation of the enzyme arginase II (Arg2) (99), which produces L-ornithine in addition to urea. This arginase induction plays 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 L-Arg level in culture medium, and bacterial killing requires high levels of L-Arg (51). Consistent with this, there is increased iNOS translation and NO production with inhibition of arginase, siRNA knockdown of Arg2, or in primary macrophages from Arg2$^{-/-}$ mice, and administration of an arginase inhibitor to *H. pylori-infected* mice increases iNOS protein expression and NO production by gastric macrophages. Second, Arg2 has a central role in inducing apoptosis of macrophages, which results from the metabolism of its product, L-ornithine, into polyamines (99). Finally, the generation of ornithine by arginase results in increased substrate for the generation of polyamines by ornithine decarboxylase (ODC), which is also induced by *H. pylori* (52,53,99), and this results in inhibition of iNOS (43). Specifically, the polyamine spermine does not alter iNOS transcription but, instead, blocks iNOS protein translation and NO production. Knockdown of ODC by RNA interference results in sufficient increases in iNOS protein expression and NO production such that killing of *H. pylori* by macrophages can be significantly enhanced (43). Although these biochemical pathways that limit NO production (summarized in Fig. 6) may protect macrophages from the potential toxic effects of

overproduction of NO in response to other pathogens, *H. pylori* appears to clearly benefit from these host responses.

Host inflammatory responses are enhanced by macrophage activation, but when there is significant apoptosis of macrophages, there can be profoundly deleterious consequences. The release of cytokines from dying cells was originally established in *Shigella* infection (317); 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 loss of host defense with the disappearance of these effector cells. For this reason, studies assessing mechanisms of macrophage apoptosis may provide important clues to persistence of *H. pylori* infection. Based on the identification that inhibition of arginase activity could block *H. pylori*-induced apoptosis, further work has shown that generation of polyamines is involved (Fig. 7). 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 spermine oxidase (originally known as polyamine oxidase 1) to spermidine, with the byproduct of this metabolism being hydrogen peroxide (H_2O_2). SMO is upregulated by *H. pylori* in macrophages, and its inhibition by a pharmacological inhibitor MDL 72527 or by RNA interference prevents the generation of H_2O_2 and the intrinsic pathway of apoptosis in macrophages (52). *H. pylori* also upregulates expression and nuclear translocation of c-Myc, and the binding of this transcription factor to the 5′ UTR of the ODC promoter mediates ODC transcription and associated apoptosis (53).

Another potential contributing factor in the inflammation to carcinoma sequence may be the generation of oxidative stress by the SMO pathway. Macrophages exposed to *H. pylori* products produce high levels of both intracellular and extracellular H_2O_2 from this enzyme (52). Various metabolites of H_2O_2, such as hydroxyl radicals, can be highly damaging to macromolecules within cells, including DNA. Oxidative DNA damage induced by *H. pylori* has been well-documented (81), and such mutations are important in the pathogenesis of gastric cancer (276). The same pathway of generation of H_2O_2 by induction of SMO also occurs in *H. pylori*-stimulated gastric epithelial cells and is a specific cause of the oxidative stress that leads to both apoptosis and DNA damage (302).

Another potential factor that may contribute to failure of the innate immune response to eliminate *H. pylori* is avoidance of effective phagocytosis by macrophages (10,316). Although *H. pylori* can be internalized into phagosomes by macrophages, these phagosomes fuse and form “megasomes” containing large numbers of live bacteria. Additionally, *H. pylori cag*$^+$ strains that produce a functional VacA toxin prevent the fusion of phagosomes with lysosomes that is needed for bacterial killing, and this disruption of phagosome maturation is lost when cells are infected with isogenic *vacA*$^-$ mutant strains (316).

C. Dendritic Cells

Dendritic cells (DCs) are of great interest in studies of the immunology of *H. pylori* infection because they represent a critical bridge between the innate and adaptive immune responses (Fig. 8). DCs have been identified as primary responders to stimuli including bacterial products and serve an important mission as antigen presenting cells (APCs). DCs can penetrate epithelial monolayers in vitro and the intestinal epithelial barrier in vivo and can engulf bacteria directly (54,193,226). Disruption of the epithelial apical-junctional complex by *H. pylori* (16) 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, respectively (31). 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 (103). Pulsing of human DCs with intact *H. pylori* and bacterial membrane preparations results in DC maturation. Coculture of pulsed DCs with NK cells results in secretion of TNF-α and IFN-γ, and with naive T cells results in TNF-α, IFN-γ, and IL-2 secretion, indicative of NK and Th1 effector responses. Activation of T-bet expression and suppression of GATA-3 levels in T cells is also indicative of stimulation of T-cell differentiation into a Th1 phenotype (108).

H. pylori outer membrane proteins, such as Omp18 and HpaA, have been reported to induce the maturation and antigen presentation capacity of DCs (287). Blood DCs and recombinant *H. pylori* proteins have been used to demonstrate expression of MHC class II and CD83 costimulatory molecules indicative of differentiation, and a preplasmacytoid, rather than a premyeloid DC, response (287). In human blood monocyte-derived DCs, activation and maturation of DCs occur independently of the presence of the *cag* PAI and *vacA* genotypes, and activation of cytokine production occurs with Formalininactivated *H. pylori*, sonicated bacteria, or culture supernatants and may be partially LPS dependent (147). IL-12 responses are also attenuated with inhibition of bacterial internalization (108,147), indicating that phagocytosis of intact *H. pylori* by DCs is an important part of the activation of intracellular receptors. DCs interact specifically with *H. pylori* by binding of glycoconjugate carbohydrate structures to DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN/CD209). It has been suggested that the identification of DC-SIGN as a novel receptor for *H. pylori* may be essential to understanding shifting of the Th1/Th2 balance in favor of persistence of the infection (19). However, Lewis antigen expression by *H. pylori* LPS has been shown to block Th1 responses by binding to DC-SIGN on DCs, thus representing a form of immunosuppression (19).

In mice infected with *H. pylori*, DCs are recruited into the stomach as early as 6 h postinoculation, but they have virtually dissipated by day 5 (139). Whether this represents movement of these CD11c positive cells to the lymph nodes, or whether this disappearance is indicative of apoptosis or other failure to sustain the response, remains to be determined. Stimulation of mouse bone marrow-derived DCs with *H. pylori* results in phagocytosis of the bacteria and expression of proinflammatory cytokines IL-1α, IL-1β, and IL-6; however, there was only modest IL-12 expression and diminished activation of splenocyte IFN-γ secretion and cellular proliferation compared with that induced by *Acinetobacter lwoffi* (139), another pathogen that causes gastritis in mice (313). In addition, *H. pylori* secreted factors are able to inhibit *A. lwoffi*-stimulated IL-12 release (139), suggesting that there may be an impaired innate response to *H. pylori* by DCs in vivo.

H. pylori stimulates primarily an IL-10 response in blood-derived monocytes, but IL-12 responses are enhanced by CD40 ligation, which is modeled by coculture of DCs with DCs transfected with CD40 ligand (182). This activation also enhanced *H. pylori-stimulated* production of IL-23 (182), a member of the IL-12 family that shares the p40 subunit of IL-12 and promotes the proliferation of the IL-17 producing T cells (Th17), a pathway that has recently been implicated in colitis (71,166). DCs activated with *H. pylori* for 48 h exhibit an attenuated ability to induce IFN-γ production upon coculture with naive T cells compared with DCs pulsed with *H. pylori* for only 8 h, and there was a similar loss of response to CD40 ligation (182). This suggests that chronic exposure of DCs to *H. pylori* results in loss of the ability to induce a Th1 response, which may contribute to persistence of the infection. New insights have derived from a comprehensive study demonstrating that there are multiple pathways involved in the recognition of *H. pylori* and its products by DCs that include TLR2, TLR4, and TLR9, with both MyD88-dependent and -independent signaling (221).

Recent data have suggested that *Helicobacter* species colonizing extragastric sites can modify the intensity of the gastric inflammatory and injury response to *H. pylori*, perhaps via dendritic cell activation of T regulatory cells. Lemke et al. (153) precolonized mice with *H. bilis* (an intestinal *Helicobacter)* or medium alone 2 wk prior to challenge with *H. pylori* (153). The severity of gastritis, atrophy, metaplasia, and hyperplasia was significantly attenuated in dual-infected mice compared with mice infected with *H. pylori* alone. This was accompanied by dampened Th1 responses to *H pylori* in coinfected animals. Recently, an independent group demonstrated that coccoid forms of *H. pylori* are phagocytosed by intestinal dendritic cells in Peyer's patches, which led to priming of $CD4^+$ T cells that subsequently migrated to the stomach and initiated gastric inflammation (189). A working hypothesis to coalesce findings from these two studies is that in *H. bilis*-infected mice, *H. bilis* antigens are processed by DCs in Peyer's patches which, in turn, prime T regulatory cells. These activated TRegs may then migrate to the stomach and inhibit *H pylori*-induced inflammation.

D. T Cells

Early studies established the concept that gastric lymphocyte populations from *H pylori-infected* patients contain increased IFN-γ-producing T cells, consistent with a Th1 cytokine response (30), and *H. pylori-specific* T cell clones derived from gastric mucosa also have a Th1-profile in patients with peptic ulcer disease (69,70). Mucosal T cells harvested from *H. pylori-infected* persons produce abundant levels of the Th1 cytokines IFN-7 and IL-2 and low levels of the Th2 cytokines IL-4 and IL-5 (30) Additional work demonstrated the importance of IL-12 production that may be derived from monocytes, macrophages, or DCs in the induction of Th1 lymphocyte response (103,108) and the role of gastric epithelial cells as antigen presenting cells in activation of $CD4^+$ Th cells (312).

Because of the consistent identification that, like Crohn's disease, *H pylori* infection induces a Th1-skewed response, this reaction is considered to be fundamental for pathological inflammation and may, in fact, be unnecessary for a pathogen that is "noninvasive." However, evidence has accumulated to suggest that the opposite may be true, in that Th1-mediated responses are of value to the host in regulating infection, but the defect is that the response is not vigorous enough. Murine studies have shown that Th1 responses are associated with increased gastritis, since IFN-$\gamma^{-/-}$ mice have decreased levels of gastric inflammation (4) and SCID mice lacking T and B cells that are infected with *H. pylori* require adoptive transfer of $CD4^+$ T cells for gastritis to develop (74). These studies have also shown that an insufficient Th1 response is associated with increased bacterial colonization (4,74), suggesting that the development of a strong Th1 response can attenuate *H. pylori* colonization (74). 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 (72).

These findings indicate that other T-cell populations are important. IL-17 has been linked to chemokine-mediated neutrophil infiltration, and IL-17 levels are increased in *H. pylori*-infected human (158) and mouse (242) gastritis tissues. Recently, it has been shown that immunization of mice with *H. pylori* lysate markedly enhanced 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 and the attenuated cytokine/chemokine response in unimmunized mice suggest that the IL-17/Th17 response may thus be defective in a normal host, thereby contributing 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 β_2-integrin receptor subunit (CD18) and utilizing integrin receptors to cause cellular vacuolization (247).

Additional investigations have implicated Tregs in the pathogenesis of *H. pylori* infection. Circulating memory T cells from *H. pylori*-infected humans have been shown to have less proliferation and IFN-γ production in response to *H. pylori*-pulsed DCs than T cells from naive donors, a defect that could be abrogated by depletion of $CD4^+CD25^{high}$ regulatory T cells. These data indicate that *H. pylori*-specific Tregs suppress memory T-cell responses and could thus contribute to the persistence of the infection (156). It has been reported that *H. pylori*-infected individuals have increased levels of $CD4^+CD25^{high}$ T cells in the gastric and duodenal mucosa that express mRNA of FOXP3, a gene involved in the development of Tregs, and high levels of the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) protein. This same study also showed more $CD4^+CD25^{high}$ T cells in *H. pylori*- associated gastric adenocarcinoma tissues than the adjacent tissue (155). Along with gastric cancer, differences in the number of regulatory T cells have been identified in patients with and without peptic ulcer disease. Robinson et al. (231) reported that *H. pylori*-infected persons with peptic ulceration had significantly less gastric regulatory T cells but increased Th1 and Th2 responses compared with infected subjects without ulcers, suggesting that imbalances within the regulatory T-cell network may predispose to diseases that develop within the context of *H. pylori* infection.

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 increased gastritis, infiltrating $CD4^+$ T cells, and production of IFN-γ than in mice receiving nondepleted lymph node T cells (223). BALB/c mice infected with *H. pylori* or *H. felis* and treated with anti-CD25 antibody to deplete Tregs developed a Th2-type response characterized by serum IgG1 antibodies and production of IL-4 and IL-5 in paragastric lymph node-derived T cells that were activated ex vivo with *H. pylori* (140). These data suggest that the Treg response can act to enhance Th1 commitment, but this study did not detect any difference in colonization levels or severity of gastritis (140). Administration of antibody to CTLA-4 during the first week of infection in C57BL/6 mice modestly decreased gastritis scores and increased the *H. pylori*-specific IgG1/IgG2a levels in the serum and the IL-4/IFN-γ ratio in splenocytes stimulated with *H. pylori* antigen (291). The anti-CD25 monoclonal antibody PC61 has been shown to deplete $Foxp3^+$ T cells and results in increased severity of gastritis, gastric cytokine levels, and serum IgG1 and IgG2c levels as well as decreased bacterial colonization in C57BL/6 mice (222). Combined with the findings that *H. pylori*-infected patients express increased levels of FOXP3 mRNA and protein in gastric lymphocytes (222), this study suggests that induction of the Treg response contributes to an equilibrium between the host and the bacterium allowing *H. pylori* to survive, but also preventing destructive inflammation.

Activation of T cells by specific antigens involves expression of costimulatory molecules, and CTLA-4 acts to inhibit this process. In the case of *H. pylori* infection, functional inactivation of $CD4^+$ T cells recruited to the gastric mucosa could be related to expression of CTLA-4 on the T-cell surface and prevention of costimulation when APCs engage T-cell receptors (18). Blockade of CTLA-4 results in increased T-cell activation in vitro and in vivo and decreased colonization in *H. pylori*-infected mice, suggesting that there is induction of anergy in $CD4^+$ gastric T cells (18). *H. pylori* can inhibit lymphocyte proliferation, and this effect as well as inhibition of IL-2 secretion by T cells has been attributed to a downregulatory effect of *H. pylori* VacA on the activation and nuclear translocation of the transcription factor NFAT (37,95). In addition, VacA has been shown to activate a MAPK signaling pathway that results in activation of the GTPase Rac leading to disruption of the cytoskeleton due to actin rearrangement (37).

E. B Cells

There is ample evidence that B cells also contribute to the immunopathogenesis of *H. pylori* infection. In studies conducted in B cell-deficient (μMT) mice infected with *H. pylori*, when compared with wild-type mice, there was no difference in colonization at 2 wk after infection, but a 2 log-fold reduction developed at 8 and 16 wk postinoculation, that was associated with increased gastric inflammation and infiltration of $CD4^+$ T cells (5). While IgG and IgA responses to *H. pylori* in the serum and gastric mucosa may be involved in protective immunity, the latter study, and another by the same group implicating the negative effect of IgA antibodies (6), suggests that B cell-mediated antibody responses may be counterproductive.

Persons infected with *H. pylori* not only have an increased risk for gastric adenocarcinoma, but they also have a significantly increased risk of developing mucosa-associated lymphoid tissue (MALT) lymphoma and non-Hodgkin lymphoma of the stomach, lesions that are composed of transformed B cells. In pioneering work, Woth-erspoon et al. (299) first demonstrated that T cells can react with *H. pylori* antigens and produce cytokines such as IL-2 that support the uncontrolled growth and proliferation of B lymphocytes, leading to lymphomatous degeneration. Although MALT lymphomas are rare in the United States, successful elimination of *H. pylori* leads to complete regression of these tumors in >80% of cases, a remarkable demonstration that removal of a bacterium can affect a clonal lesion.

Based on the seminal work of Wotherspoon et al. (299), a major focus of recent investigation has been related to the development of MALT lymphoma. Naive mouse splenocytes exposed to *H. pylori* are protected from spontaneous apoptosis and undergo proliferation in response to low, but not high, multiplicity of infection, and the responding cells are derived from the B-cell population (42). Furthermore, chronic gastric infection with *H. pylori* protects splenic B cells from apoptosis, indicating a B-cell activation/survival phenotype that may have implications for MALT lymphoma (42). In addition to producing antigen-specific antibodies, B cells have also been shown to produce autoreactive antibodies that may be pathogenic (305). The role of T cell-B cell interactions in the pathology of the immune response is an area of future investigation.

VIII. ADDITIONAL CONSIDERATIONS IN INFLAMMATION-INDUCED GASTRIC CARCINOMA

Chronic inflammation that develops in response to *H. pylori* undoubtedly contributes to transformation. Studies in *H. felis*-infected mice have demonstrated that bone marrow-derived cells (BMDC) home to and engraft in sites of chronic gastric inflammation, particularly within foci where tissue injury induces excessive apoptosis, which overwhelms the population of endogenous tissue stem cells (125). Within the inflammatory environment of the infected stomach, BMDC degenerate into adenocarcinoma, suggesting that gastric epithelial carcinomas can originate from marrow-derived sources (125). Another intriguing issue has been the association of the proinflammatory cytokine IL-1β with gastric cancer. Not only have IL-β and other proinflammatory cytokines been found to be upregulated in gastric cancer, but IL-1β polymorphisms have specifically been correlated with increased cancer risk in *H. pylori*-infected humans (76,77,82). Of note, these relationships have not been identified in all populations tested, and some studies, particularly from the Far East, have failed to demonstrate a significant association between high-expression IL-1 alleles and gastric cancer (14). These discordant results may reflect differences in the distribution of "at-risk" alleles, and future work is needed in this area (14). However, transgenic mice overexpressing human IL-1β in parietal cells develop spontaneous gastritis and dysplasia after 1 year of age and exhibit increased dysplasia and carcinoma when infected with *H. felis*

(277). Importantly, these findings were linked to activation of myeloid suppressor cells (MDSCs) through an NF-κB dependent, but lymphocyte-independent mechanism (277). MDSCs are Gr-1^{+}CD11b $^{+}$ immature myeloid cells that have been associated with tumor development and IL-1β (41). This new link to the proinflammatory cytokine IL-1β in *H. pylori* infection provides further evidence supporting a multifactorial model for the immune response against *H. pylori* and indicates that further investigation in the area of gene polymorphisms could provide new insights into the role of innate immunity in *H. pylori*-associated gastric carcinogenesis.

IX. CONCLUSIONS

Establishment of *H. pylori* as a risk factor for gastric cancer permits identification of persons at increased cancer risk. Infection with this organism, however, is extremely common, and most colonized persons never develop cancer; therefore, techniques to identify high-risk subpopulations must utilize other biological markers. Analytical tools now exist including genome sequences (*H.pylori* and human), measurable phenotypes, and practical animal models, to address key unanswered questions regarding mechanisms through which the host innate immune response to particular *H. pylori* strains drives car-cinogenesis. Since both strain genotypes and induced host responses likely influence cancer risk by differentially affecting the innate immune response, delineation of pathways activated by *H. pylori*-human interactions will not only improve our understanding of gastric carcino-genesis, but will also facilitate identification of potential therapeutic targets for prevention and more effective treatment of this disease.

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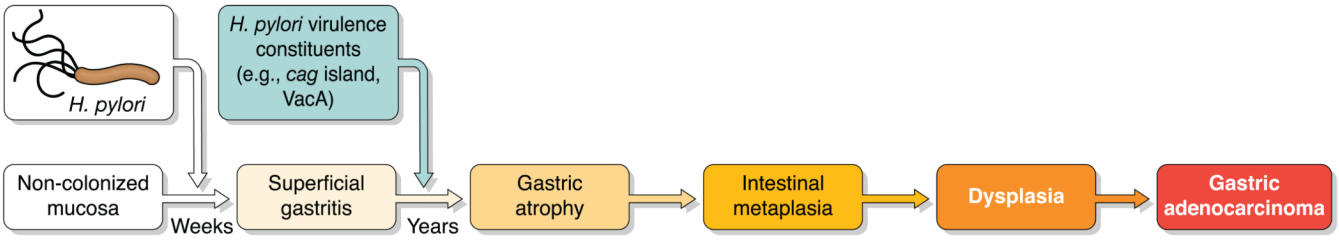

FIG. 1.

Progression to intestinal-type gastric adenocarcinoma. *Helicobacter pylori* colonization typically occurs during childhood and leads to superficial gastritis. The presence of genes such as the *cag* island and *vacA* that encode bacterial virulence factors augment the risk for progression to gastric atrophy and gastric adenocarcinoma.

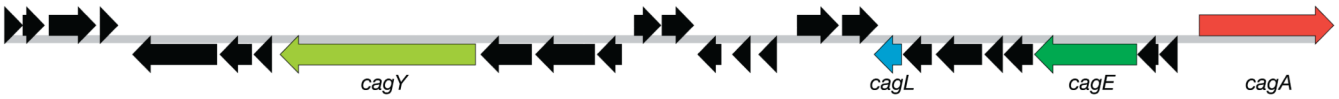

FIG. 2.

The *H. pylori cag* pathogenicity island. The *H. pylori cag* island encodes a type IV secretion system that protrudes from the bacterial surface and is induced upon contact with host cells. The *cag* pilus is covered on its surface by CagY and CagL. CagY is a large protein that contains two transmembrane domains, and CagY can differ in size due to in-frame deletions or duplications resulting in reduced host antibody recognition that may allow immune evasion. CagL binds to $\alpha_5\beta_1$-integrins on host cells to facilitate translocation of CagA. CagA is present at the tip of the pilus, and delivery of CagA into host cells proceeds in an energy-dependent manner driven by NTPases such as CagE.

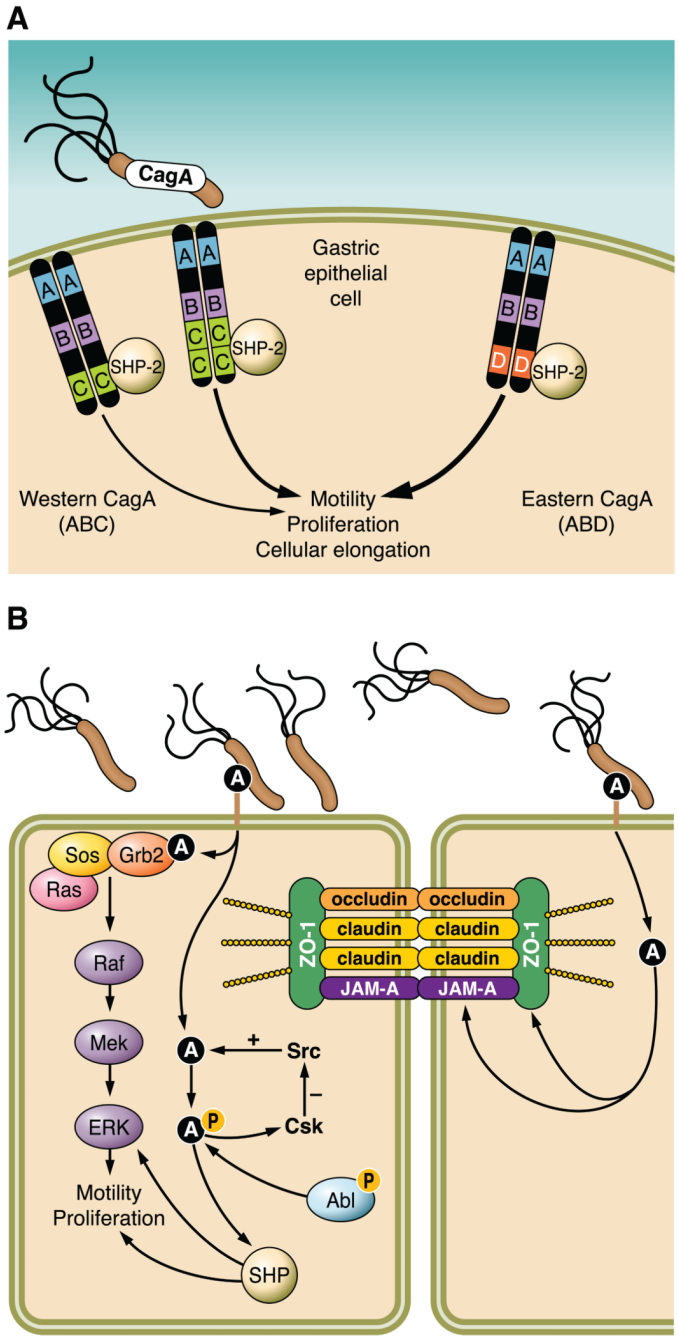

FIG. 3.

CagA phosphorylation motifs and cellular morphogenic alterations induced by intracellular CagA. *A*: tyrosine phosphorylation of EPIYA sites within the COOH terminus of CagA leads to alterations in host epithelial cells. Variation in the number and sequence of these sites determines the degree of CagA phosphorylation and the intensity of cellular changes. *H. pylori* strains colonizing individuals in Western countries typically have Western-type CagA (C) motifs, whereas those from East Asia have Eastern-Asian CagA (D) motifs. *B:* CagA (depicted as "A") is phosphorylated by Src and Abl kinases, which is followed by a decrease in levels of phospho-CagA via the inhibitory kinase c-src kinase (csk). Phosphorylated CagA activates SHP-2 and ERK also leading to cellular morphological

changes. Unphosphorylated CagA associates with the tight junction proteins ZO-1 and JAM-A leading to dysregulated apical junctional complexes. Unmodified CagA can also lead to changes in motility and proliferation through binding Grb2 and activation of the Raf/Mek/Erk pathway.

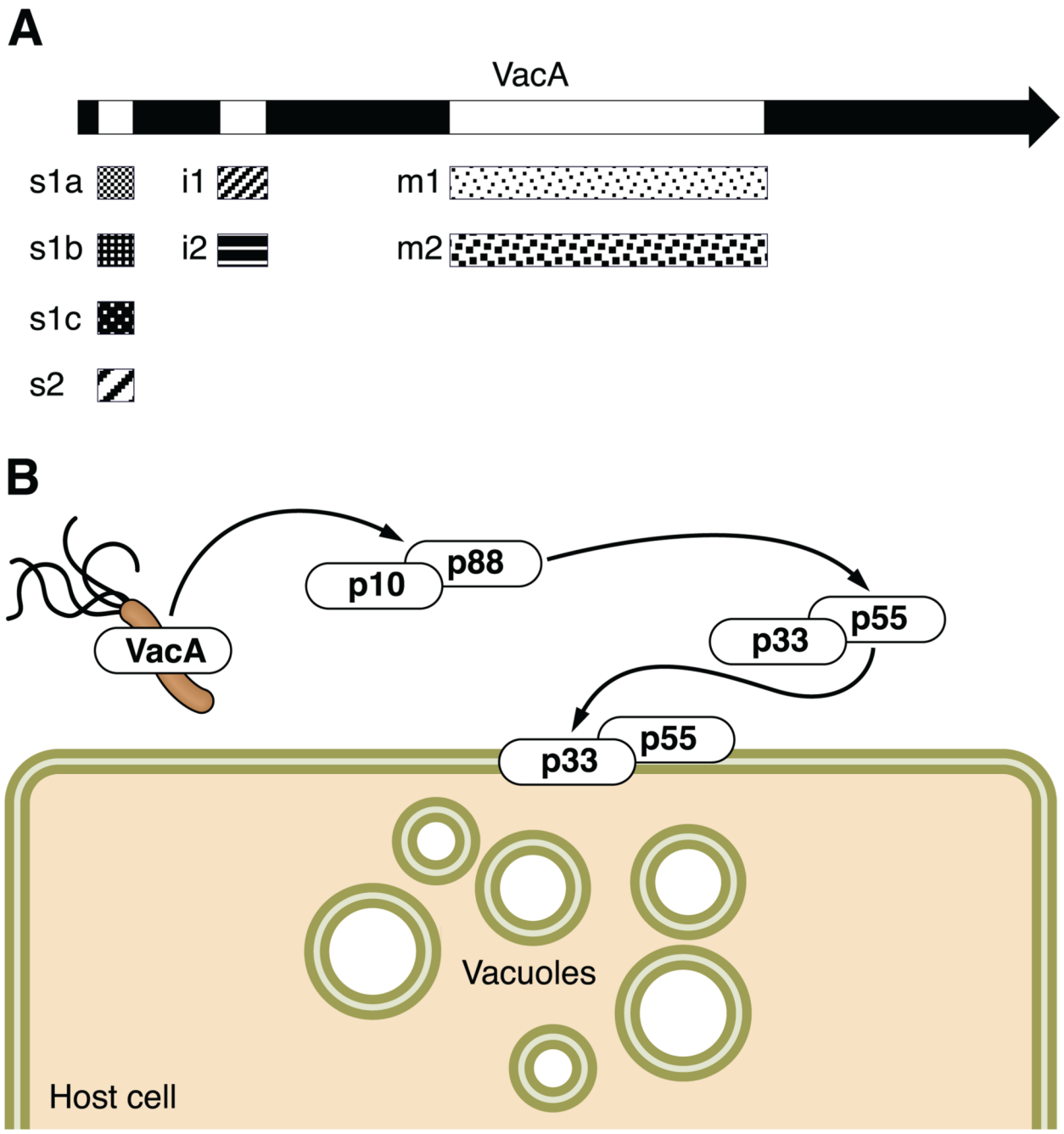

FIG. 4.

Structure of VacA. *A*: allelic diversity of *vacA* is found near the 5′ end (types s1a, s1b, s1c, and s2), the intermediate region (types i1 and i2), and the mid-region (types m1 and m2). *B*: VacA is secreted and cleaved to yield an 88-kDa protein (p88) and a 10.5-kDa protein (p10). p88 is further processed to yield two functional fragments, p55 and p33, which function in cell binding and pore formation, respectively.

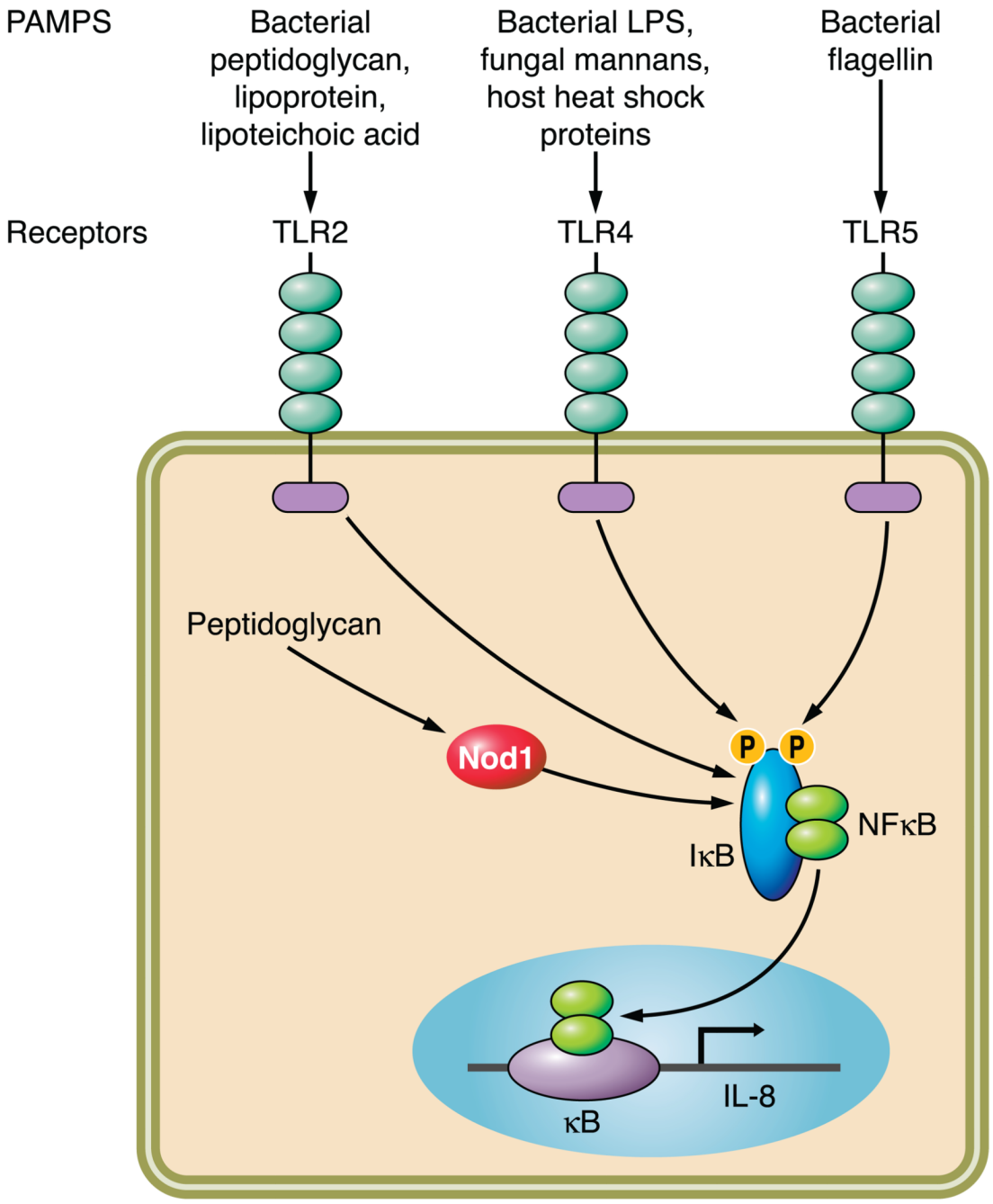

FIG. 5.

Toll-like receptors and pathogen recognition. Activation of Toll-like receptors (TLRs) and intracellular receptors (e.g., Nod1) by pathogen-associated molecular patterns (PAMPs) triggers multiple intracellular signaling pathways that culminate in NF-κB activation and subsequent production of inflammatory and immune effectors, such as interleukin-8.

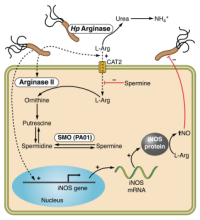

FIG. 6.

Regulation of inducible nitric oxide (NO) synthase (iNOS) synthesis and NO production. Pathways involved in the regulation of macrophage iNOS synthesis and NO production in response to *H. pylori* are depicted, in conjunction with the proposed pathogenic role of the generation of the polyamine spermine by induction of arginase and ODC that results in inhibition of iNOS protein translation.

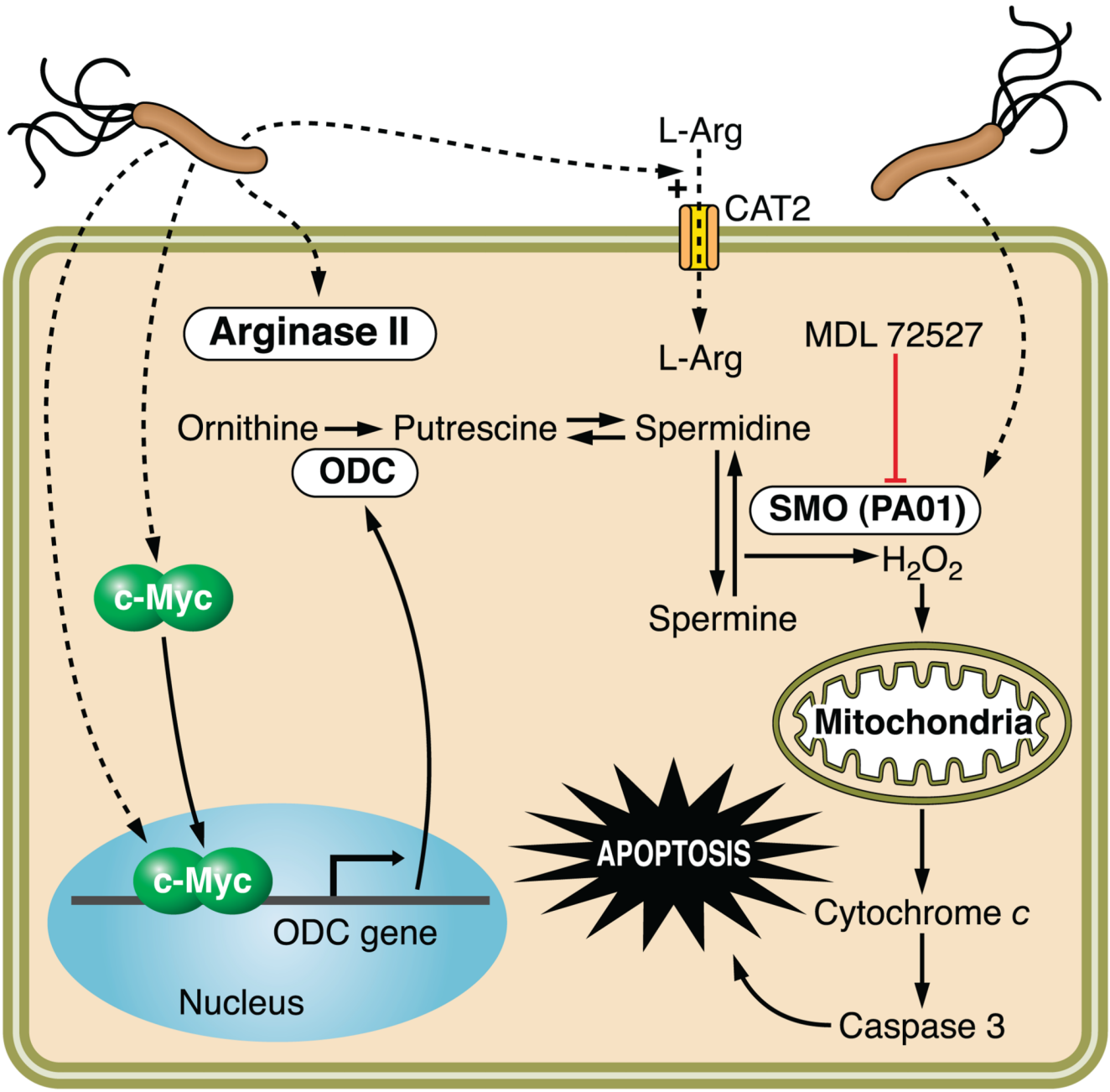

FIG. 7.

Model depicting mechanisms involved in the induction of macrophage apoptosis by *H. pylori*. *H. pylori* induces macrophage apoptosis through the generation of polyamines and increased expression and nuclear translocation of c-Myc.

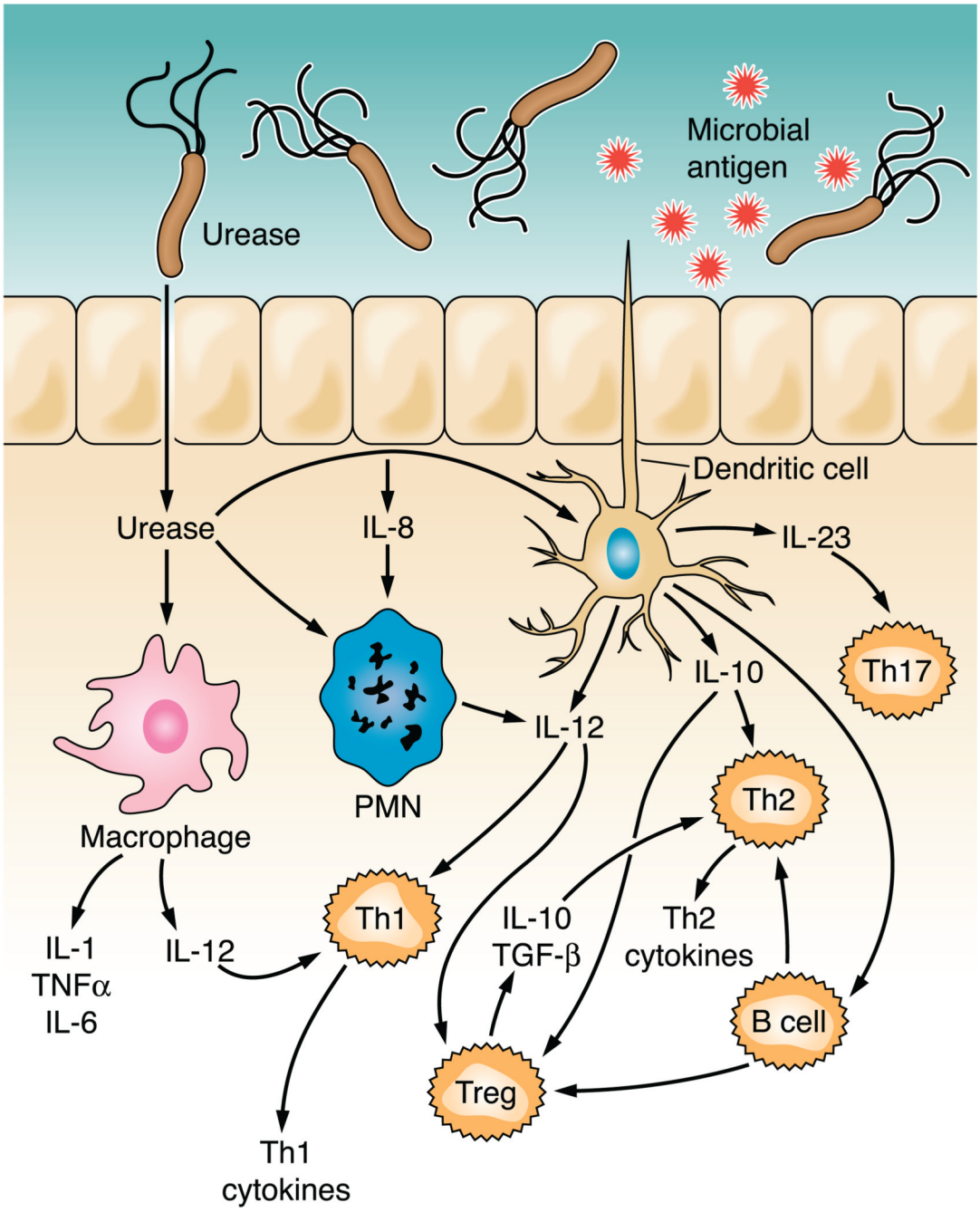

FIG. 8.

Schematic demonstarting how dendritic cells may bridge the innate and adaptice immune response directed against *H. pylori* within the gastric mucosa. Dendritic cells can penetrate the epithelial barrier in vivo and sample *H. pylori* antigens directly. Dendritic cells, in turn, activate T cells in different ways, being capable of inducing either a Th1, Th2/regulatory T cell (Treg), or a Th17 response by generation of interleukin (IL)-12, IL-10, or IL-23, respectively. Direct interactions between *H. pylori* and gastric epithelial cells or *H. pylori* constituents such as urease can also activate polymorphonuclear (PMN) cells and/or macrophages, which further amplifies the T-cell response to this pathogen.

TABLE 1

Barriers to gastric colonization

Host Barrier	***Helicobacter pylori* Avoidance Strategy**
Peristalsis	Motility
	Adherence to gastric epithelium via OMPs
Acidity	Urease production
Immune clearance	Anergic LPS and flagella
	Decreased production of HSPs
	Intracellular and intramucosal residence
	Membrane engraftment of cholesterol
	VacA-induced T-cell suppression

OMPs, outer membrane proteins; LPS, lipopolysaccharide; HSPs, heat shock proteins.