A role for altered phagosome maturation in the long-term persistence of *Helicobacter pylori* infection

Glenn N. Borlace, Stacey J. Keep, Mark J. R. Prodoehl, Hilary F. Jones, Ross N. Butler, and Doug A. Brooks

Mechanisms in Cell Biology and Disease Research Group, School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, South Australia, Australia

Submitted 16 August 2011; accepted in final form 7 May 2012

Borlace GN, Keep SJ, Prodoehl MJ, Jones HF, Butler RN, Brooks DA. A role for altered phagosome maturation in the long-term persistence of *Helicobacter pylori* infection. *Am J Physiol Gastrointest Liver Physiol* 303: G169–G179, 2012. First published May 10, 2012; doi:10.1152/ajpgi.00320.2011.—The vigorous host immune response that is mounted against *Helicobacter pylori* is unable to eliminate this pathogenic bacterium from its niche in the human gastric mucosa. This results in chronic inflammation, which can develop into gastric or duodenal ulcers in 10% of infected individuals and gastric cancer in 1% of infections. The determinants for these more severe pathologies include host (e.g., high IL-1β expression polymorphisms), bacterial (e.g., *cytotoxicity-associated gene* (*cag*) pathogenicity island), and environmental (e.g., dietary nitrites) factors. However, it is the failure of host immune effector cells to eliminate *H. pylori* that underlies its persistence and the subsequent *H. pylori*-associated disease. Here we discuss the mechanisms used by *H. pylori* to survive the host immune response and, in particular, the role played by altered phagosome maturation.

chronic inflammation; gastric cancer; macrophage; phagocytic killing; ulcer

*Helicobacter pylori* is a motile, spiral-shaped, microaerophilic bacterium that primarily colonizes the human stomach and may also be found in areas of gastric metaplasia in the duodenum (145). *H. pylori* is one of the most successful human pathogens: it is estimated to have infected >50% of the world’s population, with varying rates of infection in different countries (49). For example, in Australia, the overall seroprevalence of *H. pylori* is 15% (101); in the developing world, rates as high as 90% have been reported (87). Higher prevalence is correlated with a variety of factors, including increased age, low socioeconomic status, and poor sanitation (31). *H. pylori* causes a range of gastric pathologies and is responsible for 70–75% of gastric ulcers, 90–95% of duodenal ulcers (54), and, as the principal etiological agent for gastric cancer, 5.5% of all cancer in humans (26, 57, 115).

While *H. pylori* elicits a strong innate and adaptive immune response in all infected individuals, this fails to clear the infection (5, 142). The long-term persistence of infection results in chronic stimulation of the inflammatory immune response, which underlies all *H. pylori*-associated disease. The standard treatment regimens for *H. pylori* infection are triple and quadruple therapies that are administered over 1 or 2 wk (93). However, the incidence of treatment failure has steadily increased over the last two decades, reaching 20% in many populations (62). This is reportedly due to two factors: 1) poor patient compliance due to the 1- or 2-wk therapies and 2) increasing rates of *H. pylori* antibiotic resistance worldwide (34, 62).

We have reviewed the mechanisms used by *H. pylori* to avoid elimination by the host and, in particular, immune effector cells. Determination of the mechanism by which this bacterium evades immune killing has the potential to identify new approaches for therapeutics to clear *H. pylori* infection from the host.

Colonization of the Gastric Niche

To colonize the human stomach, *H. pylori* must first overcome two important physical barriers: stomach acid and the gastric mucous layer. The low-pH environment in the human stomach plays a vital role in preventing bacterial growth and colonization. *H. pylori* urease is thought to buffer the acid in the lumen of the stomach, through the hydrolysis of urea to bicarbonate and ammonia, thereby creating a neutral microenvironment around the bacterium (39, 99). Urease activity is coupled with an acid-gated urea channel, which enables *H. pylori* to maintain a neutral cytoplasmic pH on exposure to acidic conditions (148). *H. pylori* that have survived the acid in the stomach are then able to negotiate the thick gastric mucous layer, courtesy of their spiral shape and multiple polar flagella (108, 150). After traversing the mucous layer and reaching the gastric epithelium, *H. pylori* is believed to avoid being flushed away during the continual replenishment of the gastric mucosa by using a range of adhesins to tightly attach to the cells of the gastric epithelium (109). This allows *H. pylori* to escape the acidic conditions in the lumen of the stomach and gain access to gastric epithelial cells, which may act as a source of essential...
nutrients following vacuolating cytotoxin A (VacA)-dependent cell permeabilization (139).

Acute Inflammatory Immune Response

Through animal models and human trials, it has been established that primary infection with *H. pylori* results in the host mounting an acute inflammatory immune response that develops into chronic gastritis (27, 40). The acute inflammatory immune response is initiated following contact between *H. pylori* and gastric epithelial cells. *H. pylori* urease, cytotoxin-associated gene (cag) A (CagA), and the cag pathogenicity island (cagPAI) stimulate rapid activation of the transcription factors NF-κB and activator protein (AP) 1 (19) and induction of the proinflammatory chemokines IL-8, growth-regulated oncogene-α (GRO-α), regulated on activation normal T cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1α, and MIP-3α (28, 106) (Fig. 1). Neutrophils, monocytes, macrophages, and dendritic cells, which are recruited to the gastric mucosa, then escalate inflammation through secretion of the proinflammatory cytokines IL-1β, IL-8, and TNFα (5, 28, 71, 77, 83, 107) (Fig. 1). In a mouse model, neutrophils and eosinophils accounted for the first wave of infiltrating innate immune effector cells, with increased numbers at 8 wk and then at 26 wk after experimental infection, while a second wave of infiltrating macrophages was recorded at 26 wk after infection (6, 121). In a challenge model utilizing human volunteers, the number of lymphocytes and monocytes in *H. pylori*-infected gastric mucosa increased 2 wk after infection. This was followed 4 wk after infection by increased numbers of CD4+ and CD8+ T cells, signifying the start of an adaptive immune response (63). The importance of T cells in the immune response to *H. pylori* was shown in another human trial, in which the apparent clearance of acute *H. pylori* infection in some individuals was associated with circulating T cells (1).

Adaptive Immune Response

Macrophages and dendritic cells located in the lamina propria of the gastric mucosa play an important role in antigen presentation and initiation of the adaptive immune response to *H. pylori* (83, 138, 146). For example, *H. pylori* has been shown to stimulate the release of IL-6 from macrophages in a process that is dependent on phagocytosis (111). IL-6 is one of the principal drivers of the adaptive immune response, regulating the ratio of T helper type 17 (Th17) cells to T regulatory (Treg) cells (24) and stimulating the differentiation of B cells into antibody-producing plasma cells (82).

The *H. pylori*-specific, cell-mediated adaptive immune response consists of a complex mixture of T helper (Th1, Th2, and Th17) and Treg cells infiltrating the inflamed gastric mucosa. The predominant T cell type in the *H. pylori*-infected gastric mucosa is the proinflammatory Th1 cell (20). Th1 cells promote inflammation and tissue damage principally through IFNγ and IL-12 (133). Th1 effects can be modulated by the characteristic Th2 cytokine IL-4, which appears to downregulate IL-12 production (116), and *H. pylori* directly stimulates gastric epithelial cells to produce thymic stromal lymphopoi etin, inducing a dendritic cell-mediated Th2 response (79). Recently, the importance of Th17 cells and the Th17-to-Treg ratio in mucosal damage and immune escape has been established. Skewing the Th17-to-Treg ratio toward a Th17-based response is critical to the development of a vaccine-mediated clearance but also increases the amount of inflammation-based damage, whereas skewing the ratio toward a Treg response is required for *H. pylori* immune escape and tolerance (73).

In addition to the T cell-mediated adaptive immune response, a strong antibody response to *H. pylori* is mounted in all infected individuals (41). Local and systemic antibodies have been detected in *H. pylori*-infected individuals (122, 151), and B cells specific for *H. pylori* have been detected in the
infected gastric mucosa (95). However, since it is possible to elicit protective immunity in mice that lack B cells (53, 137) and since H. pylori infection is not cleared in humans without antibiotic intervention, the humoral immune response has been considered to be an indication of infection, rather than a marker of protection (72). Furthermore, on the basis of evidence obtained using a B cell-deficient mouse model, H. pylori-specific antibodies may actually enhance colonization (3, 72).

Inability of the Immune Response to Eliminate H. pylori

Despite eliciting a strong innate and adaptive immune response, H. pylori is rarely cleared from its gastric niche, with infections known to persist for decades (142). The fact that H. pylori is able to persist in the face of a strong antibody response and a strong H. pylori-specific adaptive T cell response and in an environment characterized by the presence of large numbers of activated neutrophils and macrophages suggests that H. pylori has mechanisms to avoid cell-free, T cell-mediated, and phagocytic killing (8).

The observation that the antibody-based immune response to H. pylori is ineffective suggests that H. pylori is exquisitely adapted to avoid the plethora of antibody-based killing mechanisms, including the agglutinating effects of antibodies in the gastric mucous layer and antibody-dependent cell-mediated cytotoxicity. In addition, H. pylori has been shown to prevent complement-mediated killing by reducing opsonization (127) and blocking the assembly of the membrane attack complex (125). Finally, H. pylori can reduce agglutination by the collectin surfactant protein D, via lipopolysaccharide phase variation (76).

The H. pylori-specific T cell adaptive immune response does not eliminate the bacterium from its gastric niche, and while skewing to a Th17 response can reduce the bacterial load in animal models, it does not provide sterilizing immunity (2, 143). The effect of Th17 cells is somewhat controversial, with some reports that they reduce inflammation and others that they promote inflammation (4, 102, 131). Nevertheless, Th17 cells are instrumental in providing protective immunity and recruiting polymorphonuclear leukocytes (PMNs), bridging the gap between the innate and adaptive immune response (45). Finally, H. pylori recruits and activates large numbers of Treg cells (92), which are now seen to be critical for downregulation of inflammation and promotion of bacterial growth (73).

There is growing evidence that H. pylori can invade the gastric mucosa with the detection of whole cocoid and spiral forms of H. pylori beyond the epithelial cell layer, within the lamina propria (17, 100, 114). Moreover, these bacteria have been observed in close association with inflammatory immune cells (105) and macrophages (69). H. pylori appears to be effectively phagocytosed by neutrophils, monocytes, and macrophages in vivo (52, 69, 105, 134, 155) and in vitro (7, 16, 36). Despite this engulfment, H. pylori appears to be able to avoid intracellular killing. For example, after phagocytizing H. pylori, neutrophils become activated, but the ingested bacteria are not always eliminated (10, 123, 124), and H. pylori has been shown to survive for up to 48 h within monocytes and macrophages (14, 29, 110, 126, 130, 153). There is increasing evidence that H. pylori facilitates this survival by altering phagosome maturation to attenuate the destructive effects of the phagosome. Alteration of phagosome maturation is likely to have other effects on immune responses that are mediated from the phagosome, such as antigen presentation. Thus, strategies that restore the normal functioning of infiltrating neutrophils, macrophages, and dendritic cells could lead to 1) enhanced antimicrobial effector function of these cells and 2) potentiation of the adaptive immune response, which depends on antigen-presenting and paracrine-signaling functions.

Chronic Inflammation and H. pylori-Associated Disease

The failure of the immune response to eliminate H. pylori results in chronic inflammation of the gastric mucosa. H. pylori continually stimulates gastric epithelial cells and infiltrating neutrophils, macrophages, and other lymphocytes to release proinflammatory cytokines, which enhance the inflammatory response (60, 66, 152). H. pylori recombinant urease is a potent stimulator of IL-12 and IFNγ secretions from peripheral blood mononuclear cells (97), and when dendritic cells are cocultured with H. pylori, they secrete proinflammatory IL-6, IL-8, IL-12, IL-1β, and TNFα (65, 84) (Fig. 1). IL-12 stimulates the differentiation of naïve T cells into a Th1 phenotype (22, 42, 64). Th1 cells are proinflammatory and secrete IFNγ (20, 74). IL-6 promotes the clonal expansion of Th17 cells (24), which release IL-17, a proinflammatory cytokine previously associated with other chronic inflammatory conditions, such as rheumatoid arthritis (81), and was found to be elevated in the H. pylori-infected stomach (94). Furthermore, IL-17 has been implicated in PMN recruitment (86); thus the activities of Th17 cells bridge the gap between the adaptive and innate phagocytic processes. This PMN/Th1/Th17 response forms the basis of the chronic inflammatory immune response that is the hallmark of H. pylori infection (Fig. 1).

It is also the nature and location of the chronic infection induced by H. pylori that underlie the different manifestations of H. pylori-associated disease. If the inflammation is limited to the antrum of the stomach, the resultant increase in acid production can cause gastric metaplasia and duodenal ulceration. In contrast, if the inflammation is pan-gastric, or corpus-predominant, the resultant hypochlorhydria can lead, instead, to the development of gastric ulcers or gastric cancer (18, 51, 78). The generally accepted model of gastric carcinogenesis involves a sequential progression from chronic inflammation to mucosal atrophy, metaplasia, and dysplasia (37, 38), and this is supported by the study of Wong and co-workers (149), who showed that only patients with atrophic gastritis developed cancer in a 7.5-yr prospective study. The idiosyncratic nature of the host inflammatory response also influences the outcome of infection. For example, in Western populations, high-expression IL-1β polymorphisms lead to enhanced inflammation, hypochlorhydria, and atrophic gastritis and are associated with increased risk of gastric cancer in H. pylori-infected individuals (50).

The failure of the immune response to clear H. pylori from the host perpetuates the inflammatory condition, which provides the necessary background for more serious disease outcomes. This raises the important question: How does H. pylori evade being killed by the critical immune effector cells, such as phagocytic cells, which are recruited in large numbers to the site of infection?
How H. pylori Circumvents Phagocytic Killing

Certain bacterial factors have been implicated in *H. pylori* avoidance of macrophage killing. Mutations in catalase (21), ClpP ATP-dependent caseinolytic protease and its chaperone ClpA (89), and RuvC Holliday junction resolvase (90) have resulted in reduced survival of *H. pylori* in macrophages due to an inability to avoid the destructive effects of reactive oxygen species (ROS). Similarly, *H. pylori* mutants defective in SpoT synthetase (154) were unable to survive the bacterial stress response initiated after phagocytosis by macrophages. Finally, it has been reported that the deletion of VacA (153) or urease (130) resulted in reduced survival of *H. pylori* in macrophage cell lines and that VacA and urease are implicated in disruption of the phagosome maturation process.

Neutrophils and macrophages have oxidative and nonoxidative mechanisms of phagocytic killing (103). Oxidative killing involves the generation of bactericidal metabolites of oxygen and nitrogen (Fig. 2). ROS and reactive nitrogen species (RNS) are produced in the respiratory burst, a process that occurs soon after phagocytosis by macrophages. (Modified from Refs. 80 and 147.)

Detoxification of ROS and RNS. *H. pylori* has developed a battery of antioxidant systems that enable it to avoid oxidative destruction (147) (Fig. 2). *H. pylori* superoxide dismutase (SodB) and catalase (KatA) act in sequence to detoxify superoxide anion and hydrogen peroxide to water and oxygen (117).

![Antioxidant systems of *H. pylori*.](image)

*H. pylori* uses arginase (RocF) to avoid the antimicrobial effects of macrophage-derived nitric oxides through competition for the common substrate l-arginine (61). *H. pylori* alkyl hydroperoxide reductase (AhpC) has been shown to have peroxynitrite reductase activity in vitro, which is likely to contribute to *H. pylori* resistance to RNS killing (33). The *H. pylori* neutrophil-activating protein (NapA) has two contradictory roles in *H. pylori* infection. One function of NapA is recruitment of neutrophils and monocytes to the infected mucosa and stimulation of these neutrophils and monocytes to produce ROS (55, 129). However, NapA also appears to protect *H. pylori* from oxidative stress damage, as NapA expression is upregulated in *H. pylori* strains carrying mutations in the major oxidative stress resistance factors: SodB, KatA, and AhpC (112, 147).

**Resisting vacuolar-type H+–ATPase-mediated acidification of the phagosome.** *H. pylori* encounters acid conditions inside the maturing phagosome similar to those in the stomach lumen. During normal phagosome maturation, phagosomes progressively recruit vacuolar-type H+–ATPase (vATPase) to the phagosomal membrane, which decreases pH from 6.1–6.5 in the early phagosome to 4.5 in the phagolysosome (56). It is likely that *H. pylori* resists the phagosome’s increasing proton concentration in the same way that it tolerates the high acid conditions encountered in the stomach lumen: with urease-mediated production of bicarbonate and ammonium ions (130).

**Disruption of NADPH oxidase targeting and specific granule recruitment.** *H. pylori* disrupts NADPH oxidase targeting in neutrophils, so that superoxide anions are released to the extracellular space, rather than accumulating in the phagosomal lumen (13), which also has implications for tissue damage and nutrient release. In neutrophils, after nonopsonized uptake, *H. pylori* phagosomes acquired flavocytochrome b$_559$ but did not recruit or retain p47$_{phox}$ or p67$_{phox}$ and rac2. The assembled NADPH oxidase complex appeared in patches on the cell surface. The bacterial factors responsible for this appeared to be surface-associated (13).

In a process akin to phagosome maturation in macrophages, nonoxidative killing by neutrophils involves the fusion of neutrophilic granules with phagosomes to deliver a cache of antimicrobial enzymes and peptides to the phagosome. Neutrophils have repositories of antimicrobial ROS and RNS and proteases/hydrolases stored in granules ready for release into the phagosome after vesicle fusion. The specific granule factor lactoferrin has not been located in *H. pylori* (33). The specific granule factor lactoferrin has not been located in *H. pylori* phagosomes. Metabolically active type 1 strains of *H. pylori* express a more potent form of the vacuolating cytotoxin VacA and harbor a type IV secretion system (T4SS) encoded by the cagPAI. Metabolically active type 1 strains of *H. pylori* (strains associated with more severe disease phenotypes) delay...
their uptake into macrophages and neutrophils by several minutes, whereas other strains of *H. pylori* are rapidly and efficiently internalized (9, 14). Although the innate receptors that initiate *H. pylori* phagocytosis are unknown (9), studies have shown that type 1 *H. pylori* strains utilize a novel signaling cascade during delayed phagocytosis. The type 1 *H. pylori* strains are reported to activate atypical PKCζ to regulate local actin polymerization on the forming phagosomal membrane, thereby avoiding PKCα-mediated activation of the respiratory burst (11, 12) (Fig. 3). Regulation of bacterial uptake via this mechanism is thought to assist in the extended survival of type 1 *H. pylori* strains in phagosomes (9). Extension of the time before internalization could give type 1 *H. pylori* strains the opportunity to synthesize and deliver proteins (into cells) that are required to enable survival within phagosomes.

**Disruption of phagosome maturation.** Phagosomes normally mature through a series of sequential fusion and fission interactions with early endosomes, late endosomes, and lysosomes, gaining and losing markers characteristic of these compartments (46, 47). The phagosome maturation process is further characterized by decreasing luminal pH due to the accumulation of hydrogen ions transported by vATPase proton pumps on the phagosomal membrane. *H. pylori* retains the early phagosome marker coronin 1 (TACO), despite gaining markers of the early endosomes Rab5 and early endosome antigen 1 (EEA1). TACO, Rab5, and EEA1 are retained on the phagosome, while Rab7 and CD63 are recruited. EEA1 tethers phagosomes arranged in close apposition, enabling homotypic fusion to form megasomes. Individual phagosomes and megasomes retain TACO, EEA1, Rab7, and CD63 while continuing to recruit lysosomal-associated membrane protein (LAMP)-1 and LAMP-2.

**Fig. 3.** *H. pylori* phagosome maturation. *H. pylori* utilizes atypical PKCζ to delay phagocytosis, allowing time for synthesis/delivery of components necessary for disruption of phagosome maturation. In neutrophils, NADPH oxidase is assembled at the cell surface, externalizing ROS. RocF outcompetes iNOS for the common substrate arginine, reducing RNS inside the cell and producing urea, which is converted to ammonium by urease, thereby nulling the effects of increasing H⁺ concentrations from the progressive accumulation of vacuolar H⁺-ATPase (vATPase) pumps on the phagosome membrane. *H. pylori* retains the early phagosome marker coronin 1 (TACO), despite gaining markers of the early endosomes Rab5 and early endosome antigen 1 (EEA1). TACO, Rab5, and EEA1 are retained on the phagosome, while Rab7 and CD63 are recruited. EEA1 tethers phagosomes arranged in close apposition, enabling homotypic fusion to form megasomes. Individual phagosomes and megasomes retain TACO, EEA1, Rab7, and CD63 while continuing to recruit lysosomal-associated membrane protein (LAMP)-1 and LAMP-2.
endosome antigen 1 (EEA1), did not become fully acidified, and only acquired limited amounts of lysosomal-associated membrane protein (LAMP)-1 (153). In one such study, *H. pylori* phagosomes acquired and retained EEA1 and Rab7, despite also acquiring the late endosome/lysosome membrane markers CD63, LAMP-1, and LAMP-2 (30). Thus it appeared that *H. pylori* compartments underwent normal fusion reactions with endosomes, but there was aberrant recovery of these components during the fission stage (Fig. 3).

Several groups have reported abnormal phagosomes containing multiple bacteria in *H. pylori*-infected macrophages (14, 30, 126, 130, 153). These communal compartments, alternately termed “megasomes” (14, 30, 130, 153), or large communal vesicles (LCVs) (126), are believed to arise due to the homotypic fusion of phagosomes containing a single bacterium (Fig. 3). LCVs reportedly differ from meagosomes, as they contain internal membranes resembling giant multivesicular bodies (126). The appearance of meagosomes was reported to be characteristic of type 1 *H. pylori* strains (14, 153), whereas LCVs were observed in macrophages, regardless of the *H. pylori* strain’s vacA or cag status (30, 126). The study of isogenic mutant strains of *H. pylori* has demonstrated that VacA and urease are essential for meagosome formation (130, 153). Megasomes and LCVs appear to arise 2–4 h after internalization and persist for ≥24 h (14, 30, 126, 130, 153).

EEA1 normally acts to tether early endosomes for homotypic endosome fusion and probably plays a similar role in the formation of *H. pylori* meagosomes. Thus, EEA1 attaches to phagosomes, which are transported toward the microtubule organizing center [via continued association with Rab7, Rab-interacting lysosomal protein (RILP), and the dynein-dynactin motor complex], where they come in closer contact. EEA1 would then promote homotypic phagosome fusion, to result in the creation of meagosomes (30, 130). In epithelial cells, the VacA-mediated retention of active Rab7 on the *H. pylori* vacuolar compartment promotes fusion with late endosome compartments (140). Rab7 retention on *H. pylori* phagosomes has similarly been shown in primary human macrophages (30), and this active Rab7 could facilitate the movement of phagosomes toward the perinuclear region of the cell, thereby contributing to meagosome formation.

In addition to disrupting the integrity of the gastric epithelium and invading the gastric mucosa, *H. pylori* has also been described as a facultative intracellular organism, able to enter and survive within the cells of the human gastric epithelium (48, 119). Using ultrastructural analysis (transmission electron microscopy) of human gastric biopsy specimens derived from *H. pylori*-infected individuals, several groups have described in vivo intracellular *H. pylori* (119). In vitro, *H. pylori* has been shown to invade the human gastric adenocarcinoma (AGS) epithelial cell line (15, 25, 85, 118, 120, 135, 140). Entry of *H. pylori* appears to occur via a receptor-mediated endocytic process that is reliant on interaction with host cell β1-integrin (69, 135) via a zipper-like mechanism utilizing phosphatidylinositol 3-kinase and PKC (85). Once internalized, viable bacteria have been observed in large VacA-dependent vacuolar structures, from which they can also engineer their release into the extracellular environment (15, 85, 140). This phagosome-like compartment is characterized by the presence of late endosome (Rab7) and lysosome (LAMP-1 and LIMP-1) markers (85, 140). It remains unclear whether *H. pylori*-containing vesicles in gastric epithelial cells associate with early endosomes. No colocalization of Rab5 or phosphatidylinositol 3-phosphate with the large *H. pylori*-containing vacuolar structures was observed after 24 h of infection in AGS cells (140). Nor was colocalization of Rab5 or EEA1 observed with VacA toxin-derived vesicles in HeLa cells (a cervical cancer-derived epithelial cell line) 20 h after these cells were incubated with the toxin (88). However, neither study examined earlier time points. HeLa cells treated with VacA toxin and examined (by confocal laser scanning microscopy) 30 min and 2 h later showed colocalization of EEA1 with vacuoles at 30 min but not at 2 h (58, 59). Whether colocalization occurs in gastric epithelial cells between Rab5, EEA1, and other early endosome markers and live bacteria is yet to be determined. It is clear that the formation of these large vacuolar structures, but not entry of the bacteria into gastric epithelial cells, depends on the presence of VacA toxin. AGS cells infected with VacA mutant *H. pylori* still internalized viable bacteria, and the smaller vacuoles that contained these bacteria remained positive for Rab7 and both lysosomal markers (LAMP-1 and LIMP-1) (140).

Several different strategies have been employed by intracellular pathogens to alter phagosome maturation and avoid phagocytic killing. *Mycobacterium tuberculosis* arrests phagosome maturation at an early stage: it recruits Rab5, but it blocks the recruitment of Rab5 effectors. The resultant lack of phosphatidylinositol 3-phosphate accumulation on the *M. tuberculosis* phagosome prevents its maturation into a phagolysosome with degradative capacity (70, 144). *Legionella pneumophila* establishes a replicative niche within an endoplasmic reticulum-derived compartment, through the actions of a range of effectors encoded on the Dot-Icm T4SS (132). *Coxiella burnetii* (the causative agent of Q fever) alters its intracellular compartment to resemble an autophagosome, which results in delayed fusion with lysosomes (23, 128). Although this compartment becomes acidified (pH ~4.8) and gains some lysosomal proteins, *C. burnetii* avoids phagosomal killing for sufficient time to become replicative (96). The specific virulence factors that enable it to avoid destruction are largely unidentified, but there is a great deal of interest in the genes encoded on the *C. burnetii* T4SS (56). It appears that *H. pylori* has developed a mechanism distinct from the latter processes that targets endosomal machinery, preventing the recovery and fission of these organelles during the phagosome maturation process (30).

It is evident that the immune response to *H. pylori* is ineffective, because the arsenal of antimicrobial devices employed with and without effector cells that normally eliminate the bacteria has been compromised. *H. pylori* has a series of mechanisms that enable it to evade cell-mediated and cell-free immune killing. While the bacterium has been shown to be efficiently coated by antibodies in vitro and in vivo (72, 141), *H. pylori* also avoids antibody-dependent cell-mediated cytotoxicity, complement fixation, and the agglutinating effects of antibodies. Furthermore, the effector cells recruited to the site of infection are unable to clear the bacterium by the array of antioxidant compounds that can be deployed by *H. pylori* and by disturbed phagosomal maturation. Blocking the development of a phagosome into a lethal compartment provides a protected niche for *H. pylori* in the inflamed gastric mucosa that could contribute to its long-term survival in the host.
Typically, intracellular pathogens take control of the host vesicular machinery to avoid being killed by phagocytic cells. There is evidence that *H. pylori* exerts control over the host vesicular machinery, but we do not fully understand how this is achieved. Components of *H. pylori* may sequester vesicular trafficking machinery, act on global regulators of trafficking and vesicular machinery, insert components into the phagosome membrane, or influence the phosphatidylinositol drivers of specific membrane interactions.

**A Strategy to Effect *H. pylori* Clearance by the Immune Response**

*H. pylori* vaccines have been unsuccessful in providing protection in humans. The successful vaccination protocols developed in the mouse model are unsuitable for application in humans, as they require the use of cholera toxin or cholera toxin derivatives as adjuvants (136). Importantly, the strong specific immune response that is already mounted in all infected persons merely directs *H. pylori* to immune effector cells, which are then unable to eliminate the internalized bacteria (75).

The current standard treatment regimens for *H. pylori* infection are triple therapy (incorporating amoxicillin and clarithromycin in combination with a proton pump inhibitor) and quadruple therapy (incorporating bismuth, tetracycline, metronidazole, and a proton pump inhibitor) over 1 or 2 wk (93). More complex and costly therapies have been developed for infections recalcitrant to treatment. However, quintuple therapy [triple therapy plus lactoferrin and probiotics (43, 156)], sequential triple-quadruple therapies, or therapies targeted by pretesting isolates for antibiotic sensitivity fail to address the underlying cause of increasing treatment failure, i.e., increasing antibiotic resistance. Disruption of the bacterial factors responsible for seizing control of the eukaryotic phagosome maturation process could reinstate the efficacy of the immune response and result in improved clearance of *H. pylori* infections resistant to antibiotic therapy. This type of strategy is exemplified by the targeting of nonreplicating populations of *M. tuberculosis* using rhodamine agents (32, 98). Recently, Hoy et al. (67) identified an *H. pylori* virulence factor important for bacterial invasion of the epithelia. High temperature requirement A (HtrA) cleaves E-cadherin, disrupting cell-cell adhesion, promoting intercellular entry of *H. pylori*. Previously, proteolytic cleavage of E-cadherin was linked to the malignant progression of adenocarcinomas (35, 44). By using a virtual screening approach, Lower et al. (91) identified inhibitors of HtrA that may help prevent bacterial infiltration of the epithelium.

Alternatively, a strategy to restore phagosome maturation could assist the host’s immune system to clear *H. pylori* infection. A concerted approach to eliminate the free-swimming bacteria in the mucous layer with antibiotics, targeted vaccination strategies that skew the adaptive immune response to an effective Th17-mediated response, and improvement in the efficacy of pathogen elimination by the phagocytic effector cells recruited by the inflammatory immune response could provide the multipronged attack that is clearly required to eliminate this persistent pathogen. For this novel strategy to be effective, the critical factors that *H. pylori* utilizes to disrupt the phagosome maturation process will need to be identified. Then specific drugs that target the *H. pylori* factors interfering with host trafficking machinery could correct the phagosome maturation process and restore this integral part of the host’s immune system.

**Conclusion**

Survival within phagocytes is a key component of persistent *H. pylori* infection. Phagocytes may provide a protected niche within the inflamed mucosa and a reservoir for reinfection when the delicate balance between the proinflammatory and the tolerogenic responses swings to the more effective Th17-mediated response shown to be critical for the reduction of bacterial load in immunized mice. *H. pylori*-mediated disruption of phagosome maturation could also affect the nature of the inflammatory immune response, as dendritic cells, macrophages, and neutrophils are the main controllers of the immune response in the gastric mucosa. Restoration of the normal process of phagosome maturation could therefore redirect the immune response to a more effective type of response. The key observation that *H. pylori* disrupts host cellular machinery and persists in the very effector cells recruited to eliminate it points to this process as being central to the long-term survival strategy of *H. pylori*.

The decreasing efficacy of *H. pylori* antibiotic treatment has important global significance for health and disease, as gastric cancer cases that are attributable to *H. pylori* infection account for 63.4% of stomach cancers, representing 5.5% of all cancers worldwide (115). Eliminating *H. pylori* and avoiding the onset of pathology would have profound consequences for public health, particularly in developing countries, where *H. pylori* infection is rife. In infected individuals, the immune response fails to clear *H. pylori*, despite directing it to phagocytic cells, which would normally kill bacteria. A better understanding of how phagocytosed *H. pylori* alters the critical process of phagosome maturation and circumvents the cell-mediated adaptive immune response will offer new avenues for targeted therapeutic intervention.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

G.N.B. and M.J.P. prepared the figures; G.N.B., M.J.P., and D.A.B. drafted the manuscript; G.N.B., S.J.K., M.J.P., H.F.J., R.N.B., and D.A.B. edited and revised the manuscript; G.N.B., S.J.K., M.J.P., H.F.J., R.N.B., and D.A.B. approved the final version of the manuscript.

**REFERENCES**


4. Algood HM, Allen SS, Washington MK, Peek RM Jr, Miller GG, Cover TL. Regulation of gastric B cell recruitment is dependent on
Review

H. pylori PHAGOSOME MATURATION AND PERSISTENCE

G176


