Special Section

The Biology of *Helicobacter pylori* Infection, a Major Risk Factor for Gastric Adenocarcinoma

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Abstract

***Helicobacter pylori* infection of the human stomach is the most important risk factor for development of gastric cancer. Whereas persistent viral infection leads to a number of cancers, *H. pylori* was the first bacteria linked to a human cancer. The exact mechanisms that lead to cancer induction are not clear, but study of the bacterial factors important for colonization and the host responses to the infection are starting to yield important clues.** (Cancer Epidemiol Biomarkers Prev 2005;14(8):1853–8)

Introduction

There are several features of *Helicobacter pylori* infection that likely influence its ability to cause severe disease. *H. pylori* is a very successful pathogen infecting at least 50% of the population worldwide. Infection is acquired in childhood and then often persists for the lifetime of the individual in the absence of antimicrobial therapy. The bacteria persist in spite of activation of both the innate and adaptive arms of the immune system. Interestingly, the bacteria seem to actively skew the immune response to a T-helper 1 (Th1) response, characterized by production of IFN-γ and leading to considerable cell damage. Another feature distinguishing *H. pylori* infection from infection with other less pathogenic *Helicobacter* species is its close contact with host cells. This intimate adherence facilitates the delivery of bacterial toxins that further damage and reprogram host cells. This has led to a prevailing hypothesis where persistent infection by this organism allows continual delivery of virulence factors and induction of a damage-associated immune response. These two processes lead to increased host cell turnover, providing a nutrient-rich colonization niche for the bacteria while putting the host at risk for accumulating genetic and epigenetic changes that lead to the development of cancer. In this review, we focus on recent progress clarifying the complex immune response to this infection, understanding the expression of bacterial virulence genes, and understanding how these proteins influence the host immune responses (summarized in Fig. 1).

Innate and Acquired Immune Responses to *H. pylori* Infection

Elaboration of an Adaptive Immune Response. *H. pylori* infection of the gastric mucosa induces an immune response involving innate and acquired components. Upon *H. pylori* infection, the response of epithelial cells as well as cells of the acquired immune system includes the production of several chemokines and cytokines, including interleukin (IL)-8, IL-10, IL-18, IL-12, tumor necrosis factor-α (TNF-α), and INF-γ that modulate the strength and kind of immune response activated, predominantly biased toward a Th1 immune response. Several recent studies on the chemokine/cytokine profile induced in the *H. pylori*–infected gastric mucosa, as well as the kind of immune cells recruited to the site of infection, have confirmed this bias. Wen et al. (1) have looked at the global inflammatory profile of the gastric mucosa of infected and uninfected patients using microarrays, confirming the previous notion of a Th1-driven response during natural infection of humans.

Linking the Innate and Adaptive Immune Responses. Dendritic cells have been detected in the *H. pylori*–infected gastric mucosa. These cells are the link between signals derived from pathogens and other cells of the immune system, such as natural killer cells and T cells, by a variety of mechanisms. Dendritic cells present antigens via MHC class II molecules and induce signal transduction cascades. They also produce B7 and costimulatory molecules that amplify and stabilize the immunologic synapse, modulating the amount of cytokines released, including IL-12, INF-γ, and TNF-α. Guiney et al. (2) showed that human dendritic cells respond to *H. pylori* infection by preferentially producing IL-12 (typical of a Th1 response) rather that IL-6 or IL-10 *in vitro*. Another study confirmed the production of IL-12 by dendritic cells upon *H. pylori* infection (3), but also reported increased IL-10 induction. They suggest that moderate induction of this cytokine (more typical of a Th2 response) may be important for the activation of T regulatory cells. *H. pylori*–specific regulatory T cells can suppress the memory T-cell response to *H. pylori* in infected individuals (4). Interestingly, it has been reported that a Th2 response might be induced in *H. pylori*–infected children (5), suggesting age-specific differences in the nature of the immune response to *H. pylori*. Further work on dendritic cells showed that *H. pylori*–pulsed dendritic cells can activate natural killer cells and naïve T cells *in vitro* to become Th1 effector cells and that bacterial membrane proteins likely mediate these effects (6). A final study observed that whereas *H. pylori* exposure induced apoptosis of human monocytes, dendritic cells were immune, lending further support to a model where dendritic cells play a central role in immune modulation during *H. pylori* infection (7).

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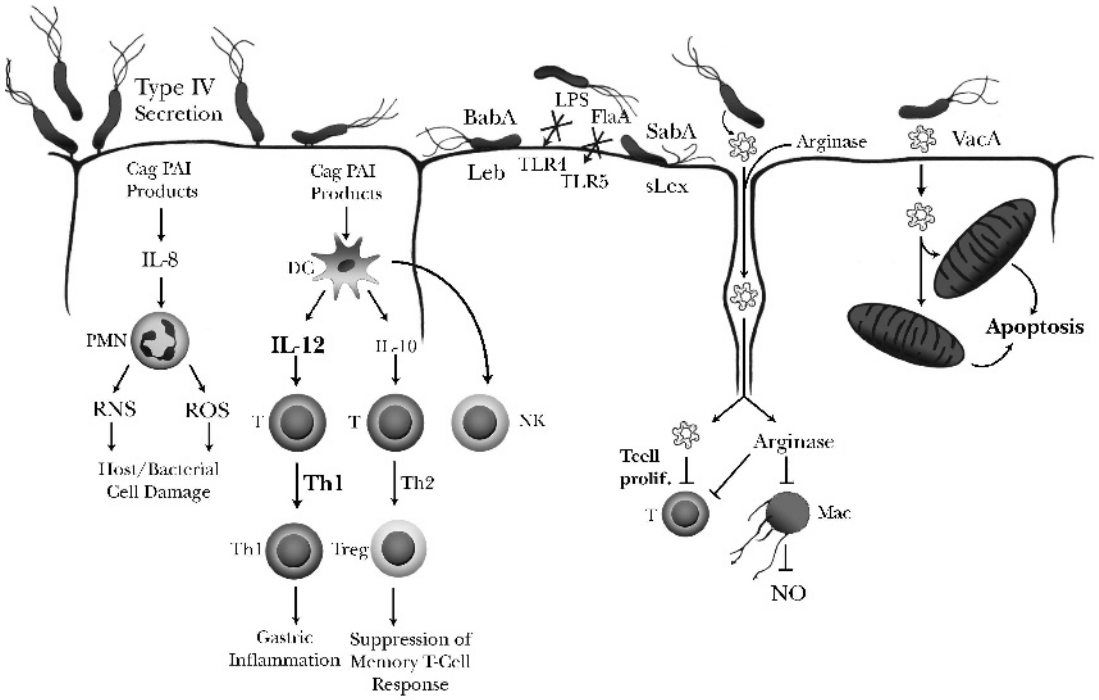

Figure 1. Innate and acquired immune responses during *H. pylori* infection. *H. pylori* infects the gastric mucosa, making intimate contact with gastric epithelial cells through molecules such as the adhesin BabA. Adhesion to epithelial cells allows secretion of virulence factors, such as CagA and VacA, into the cells, where their activity can modify host cell function. *H. pylori* establish a chronic infection despite strong induction of host immune responses. Inadequate innate immune recognition, through mechanisms such as the inability of *H. pylori* lipopolysaccharide to be recognized by Toll-like receptor 4 (TLR4) as well as the inability of FlaA to activate TLR5, might contribute to the failure of the adaptive immune response to clear the infection. In terms of the adaptive immune response, dendritic cells have emerged as an important cell population responding to *H. pylori* infection by preferentially producing IL-12 (typical of a Th1 response) and a moderate IL-10 induction (more typical of a Th2 response) that might be important for the activation of T regulatory cells. *H. pylori*–specific T regulatory cells can suppress the memory T-cell response to *H. pylori* in infected individuals, contributing to the inability of the host to clear the infection. Furthermore, other cells of the immune system are also affected by *H. pylori* products: For example, VacA has been reported to modify T-cell proliferation and S.*cag*PAI products, in addition to inducing IL-8 secretion by epithelial cells (responsible for the infiltration of neutrophils in the *H.pylori*–infected gastric mucosa), have recently been implicated in the induction of monocyte apoptosis. Apoptosis of macrophages is also induced by *H. pylori*, an activity that requires the enzyme arginase II. A role for arginase in impairment of macrophage activity and T-cell proliferation has also been described.

Suppression of the Adaptive Immune Response. It has long been recognized that *H. pylori* infection induces an active immune response and mechanisms allowing avoidance of this response have also been described. For example, *H. pylori* is able to interfere with Fas-FasL interactions leading to T-cell death (8). Recently T regulatory cells have emerged as important player in dampening *H. pylori* specific T-cell responses (4, 9). Activation of this cell population may explain how *H. pylori* persists for so long in the human stomach despite inducing a strong immune response.

Evasion of Innate Immunity. *H. pylori* also seem to be armed with mechanisms for protection against innate immune responses. Variations in the *H. pylori* lipopolysaccharide seem to account for its ability to avoid activation of TLR4. Recently, it has been reported that the main *H. pylori* flagellin subunit, FlaA, is a less potent activator of TLR5 than FliC of *Salmonella typhimurium*, both because it does not activate TLR5 well and it is not released to the media (10). Inadequate innate immune recognition of *H. pylori* might contribute to the failure of the adaptive immune response to clear *H. pylori*.

In summary, dendritic cells have emerged as a likely player in the activation of a Th1-type response that mediate gastric inflammation. Meanwhile a combination of bacterial avoidance of innate defenses and activation of T regulatory cells act to dampen immune responses and may contribute to the inability of the host to effectively clear the bacteria facilitating the chronic nature of this infection.

H. pylori Virulence Factors and their Modulation of Host Cell Responses

H. pylori expresses a number of factors to facilitate its colonization. Upon ingestion, a highly active urease enzyme produces ammonia to locally buffer the bacteria from the acidic pH in the lumen of the stomach. Next, two to six sheathed flagella allow penetration of the viscous mucous layers to the preferred niche of the bacteria in the more neutral mucous layer just overlying the gastric epithelium (11). Finally, a number of adhesins, such as the Lewis B (Leb)-binding protein (BabA), mediate attachment of a portion of the bacteria to gastric epithelial cells. This intimate attachment allows delivery of secreted molecules such as vacuolating cytotoxin (VacA), neutrophil-activating protein (NapA), and the cytotoxin-associated antigen (CagA). A major challenge in *H. pylori* research has been to elucidate when and where these and other virulence factors are expressed and their impact on host cell functions.

Regulation of Virulence. Sequencing of the *H. pylori* genome revealed a dearth of transcription factors and regulatory proteins that orchestrate the adaptation of other enteric bacteria to the complex and varied environments of the human host. This, combined with the inability to identify environmental or animal reservoirs of *H. pylori*, led to proposals that this organism has a limited spectrum of gene expression. A number of recent studies directly tested this by whole-genome transcriptional profiling of the bacteria using gene arrays. The first study queried gene transcription changes over the growth curve *in vitro* and found a dramatic shift in gene expression at the transition point between log phase and stationary phase (log/stat switch; ref. 12). They observed down-regulation of genes involved in cell growth (ribosomal proteins, transcriptional machinery, and central and intermediate metabolism) and up-regulation of genes involved in stress responses and virulence. One striking group of genes identified consists of genes involved in iron utilization. Sequestration of iron is major host defense and iron acquisition and storage is major challenge facing infecting bacteria. Iron uptake genes, like *fecA*, were down-regulated, whereas iron storage genes, like the nonheme ferritin (*pfr*) and *napA*, were up-regulated as were several iron-containing enzymes. Also up-regulated was the virulence gene *cagA*. Interestingly, another gene in the pathogenicity island (*cag1*) that has not been implicated to date in virulence was also highly up-regulated, suggesting that the role of this gene in virulence should be reexamined. Contrary to the prediction of constant gene expression for this organism, this study paints a much more dynamic picture with 40% of the genome expressed at any given time.

A follow-up study sought to directly measure the response of the bacteria to iron limitation and supplementation (13). Because of the profound differences in gene expression in log phase versus stationary phase, transcriptional response to iron limitation were measured under both conditions. Again, using microarray analysis, 138 genes showed expression changes of 2-fold or more. Many genes had growth phase–dependent responses. For example, *pfr* was only induced by iron limitation in stationary phase. Among the pathways induced nitrogen metabolism genes, particularly *amiE* and *amiF* showed dramatic changes in expression. These amidases produce ammonia, which is the preferred nitrogen source of *H. pylori*. Ammonia also causes cell damage and, therefore, could participate in release of iron sequestered in host cells. Virulence genes, including *vacA*, *cagA*, and *napA*, which acts as a bacterioferritin in addition to activating neutrophils, were also induced by iron limitation. This indicates that iron limitation may serve as a signal to the bug to turn on virulence gene expression, a phenomenon described in many other pathogenic bacteria (14). Another important observation from this study was that prolonged exposure to iron-limiting conditions (>10 hours) leads to rapid death of exponential cultures, whereas stationary phase cultures were highly resistant, suggesting that this growth state might be most relevant *in vivo*.

Two recent studies examined the global transcriptional response to moderately low pH exposure (pH 5.0; refs. 15, 16). Both studies observed down-regulation of a number of outer membrane proteins, indicating a remodeling of the surface during acid exposure. Both studies also observed strong induction of ammonia-producing enzymes as expected. The first study observed induction of type 1 flagellar genes and confirmed with video microscopy that pH 5 exposure leads to enhanced motility. This result is consistent with the observation that *in vivo* pH is the most relevant cue for chemotaxis within the mucous layer (11). Interestingly, *cagA* and *vacA* were down-regulated and the repression of *cagA* expression was confirmed at the protein level. This indicates that acid may not be a major signal for virulence gene expression.

The two studies differed with respect to expression of the iron transport accessory proteins ExbB and ExbD. Whereas the first study found this operon induced, the second study found it repressed. Whereas neither study confirmed the gene array data for these genes by an independent method, repression of these transcripts is consistent with other work demonstrating a link between low pH and metal ion regulators where low pH induces expression of the *nikR* nickel-responsive transcriptional repressor (17). NikR represses its own transcription as well as the divergent *exbBD, TonB* operon. This results in lower iron uptake and down-regulation of the Fur transcriptional regulator. In support of this, Contreras et al. (18) observed that 35 of the 101 acid responsive genes in their study were dependent on Fur for their acid regulation. Furthermore, both *fur* and *nikR* mutant bacteria were attenuated in a mouse model of infection and the double mutant had an even stronger phenotype.

Whereas the above experiments provide evidence that *H. pylori* gene expression is more dynamic than originally thought, these studies were all done *in vitro* on broth-grown bacteria. A recent study used quantitative PCR to compare bacterial gene expressions *in vivo* using fresh biopsy specimens to parallel *in vitro* cultures after 36 hours (likely in stationary phase; ref. 19). They observed a 4 log range of expression values of the 37 genes tested in six patients and very good correlation between samples. When comparing *in vitro* with *in vivo* grown bacteria, they observed a high correlation but with 5-fold lower mRNA levels in the *in vivo* samples. The one gene that fell outside the 95% confidence interval was *cagA*, and it showed even lower expression during *in vivo* growth relative to *in vitro* growth. The authors suggest that the bacteria are primarily in stationary phase *in vivo*, highlighting the importance of the log/stat switch described above.

In summary, a variety of *in vitro* and *in vivo* studies have highlighted stationary phase gene expression as most relevant to bacterial survival *in vivo.* These studies also show that whereas acid exposure may be an important cue for chemotaxis of the bacteria within the stomach, iron and/or nickel limitation may be the major signal for induction of virulence gene expression.

Oxidative Stress. One group of genes that was induced during stationary phase in the microarray experiments described above was stress response genes. A hallmark of *H. pylori* infection is infiltration of neutrophils that produce reactive oxygen and nitrogen species. There has been much interest in mechanisms the bacteria use to counter these innate host defenses. Early work showed that the major proteins that cope with reactive oxygen species in other bacteria, catalase and superoxide dimutase, are essential for survival in mouse models of infection (20, 21). Recent work has focused on DNA damage that is induced by oxidative damage in both the bacteria and the host.

On the bacterial side, O'Rourke et al. (22) showed that HP0585, one of two predicted homologues of the *Escherichia coli* Endo III, is solely responsible for repairing oxidized pyrimidine residues. *H. pylori* mutant in this gene are more sensitive to oxidative stress induced by drugs or activated macrophages. Finally, bacterial mutants in this gene are attenuated for persistence during experimental infection of mice.

On the host side, Ladeira et al. (23, 24) investigated the extent of DNA damage in normal and infected gastric tissue using a single-cell comet assay. They found that DNA damage correlated with *H. pylori* infection and degree of gastritis. Whereas there was no significant difference between males and females, there were trends toward higher damage in males. Males have a higher incidence of gastric cancer than females. Interestingly, they observed significantly more damage in those >50 years old versus those <17 years old after

controlling for degree of gastritis. A follow-up study correlated the presence of virulence genes with the degree of DNA damage, further establishing the link between highly pathogenic *H. pylori* strains and more severe disease.

These studies on the bacterial and host sides show that induction of DNA damage is an important byproduct of host defense and that the bacterium has evolved effective strategies to overcome this insult.

VacA. VacA was named for its dramatic activity on HeLa cells where it causes cell to fill with massive vacuoles derived from late endosomal membranes. Since its discovery in 1989, a vast array of activities have been linked to the molecule, including membrane insertion, anion conducting channel activity, alteration of transepithelial resistance, inhibition of antigen processing, and induction of apoptosis (25). Infection experiments in mice indicate that active toxin provides a selective advantage (26), but whether any of the reported activities are relevant during human infection remains a mystery in spite of a number of interesting recent studies.

One conundrum from the animal studies showing a colonization phenotype for *vacA* mutants was that this phenotype was discovered in a competition experiment with a mixed culture of wild-type and mutant bacteria. Because VacA is a secreted toxin, and purified toxin administered to the outside of cells reproduces all of the VacA-attributable phenotypes of intact bacteria, one would have expected the wild-type bacteria to *trans*-complement the mutant bacteria, but this was not the case. Ilver et al. (27), however, argue that the predominant mode of delivery of VacA is directly from the bacterial cell surface to epithelial cells in an elegant series of immunofluorescence experiments. A number of structure function studies have delineated residues in the amino terminal portion of the protein that are important for oligomerization and membrane insertion (28-30). One clinically relevant finding of these and other studies is that these sequences include the variable region defining the *s1* and *s2* alleles. In particular, the *s2* allele, which is correlated with less severe disease during human infection, cannot insert into membranes, induce vacuolation or, as recently shown by Cover et al. (31), induce apoptosis. Further work on the apoptotic activity of VacA revealed that this activity does not involve caspase activation or induction of the mitochondrial permeability transition, but does involve an alteration of the proton motive force that precedes cytochrome *c* release (32). A potential role for VacA oligomerization has been revealed by an elegant study demonstrating that aggregation of Rab7-containing vesicles is required for vacuolation of cells (33).

In addition to progress made in elucidating the mechanisms by which known VacA phenotypes occur, two new activities have been suggested. Most research has focused on *H. pylori* and VacA interactions with epithelial cells, but Gebert et al. (34) have now shown that purified VacA can inhibit T-cell proliferation, although the mechanisms is unclear as intact bugs seem to inhibit proliferation by VacA-independent mechanisms.

Whereas the exact mechanisms of VacA entry into host cell is still not clear, it was shown some time ago to bind protein tyrosine phosphatase receptor type Z (Ptprz; ref. 35). Recent work has shown that when purified VacA is administered to wild-type mice, they get ulcers; on the other hand, Ptprz$^{-/-}$ mice do not (36). Gastric cells cultured from the mutant animals show that Ptprz binding is not required for internalization or vacuolation by VacA, but the VacA binding to this receptor activates it and the downstream Git-1 signaling protein. Activation of this receptor by VacA causes cell migration in primary cultures of gastric epithelial cells from wild-type but not Ptprz$^{-/-}$ mice. Finally, administration of pleiotrophin, the natural ligand for Ptprz, can cause ulceration in wild-type mice.

The overwhelming theme of VacA research seems to be that this protein is playing multiple roles in disease progression and probably affects multiple cell types in unique ways.

Adherence/BabA. Another virulence gene whose role in disease has been most firmly established by epidemiology studies is the Leb-binding protein adhesion BabA. BabA is part of a family of related outer membrane proteins and displays particular homology to the BabB outer membrane protein. The *babA* gene has been found to be variably present among strains (37) and recent work has shown that it can undergo phase variation, gene conversion, and sequence variation during human infection. Screening of a panel of 39 strains with two different single-chain fragment variable recombinant anti-BabA antibodies revealed that 18 (46%) strains expressed the protein (38). Among the strains expressing the protein, there was a large range in Leb-binding affinity. Sequence analysis of high-affinity and low-affinity binding genes revealed lots of polymorphisms and no obvious correlation between sequence and binding strength. Another study examined the binding properties of an even larger panel of clinical isolates (39). They defined two different binding classes: generalists and specialists. Generalists represent most strains worldwide and bind both Leb (on O blood group individuals) and Aleb (on A blood group individuals). Specialists are almost exclusively found in South American native populations and bind Leb only. Phylogenetic analysis of variable regions of BabA shows several clusters. Specialists fall in almost all clusters. By sequence analysis of housekeeping genes, these Peruvian strains look most like European strains, which are predominantly generalists. This led to a proposal that BabA is being constantly selected for its adherence properties and that populations with predominantly blood group O lose the ability to bind Aleb. A final study sought to measure changes in BabA during the course of infection (40). Using a Rhesus macaque infection model, they found loss of BabA expression and Leb-binding activity during experimental infection with a human isolate containing *babA*. This loss could occur by recombination of a second copy of a related gene, *babB*, into the *babA* locus or by phase variation in number of CT repeats in the 5′ region of the gene, causing frameshift mutations. These data may support the idea of loss of BabA activity in the absence of strong selection for binding.

Arginase. In 2001, Gobert et al. (41) reported a role of *H. pylori* arginase on the inhibition of macrophage nitric oxide production due to depletion of L-arginine and subsequent impairment of the bacteriocidal activity of macrophages. Induction of macrophage apoptosis by activation of arginase II has also been observed upon *in vitro* incubation of macrophages with *H. pylori* (42). Recently, a new role for *H. pylori* arginase in the function of cells of the immune system has been described. Zabaleta et al. (43) have reported inhibition of T-cell proliferation and reduction of TCR CD3ξ-chain expression by *H. pylori* arginase. Knowing that L-arginine was an important modulator of T-cell function, they sought to investigate the role of *H. pylori* arginase in avoiding the immune response and found that *H. pylori* sonicates and live *H. pylori* were both able to decrease the proliferation of Jurkat T cells and freshly isolated human normal T lymphocytes. The decrease in proliferation was not due to induction of apoptosis or decrease in cell viability but rather interference with the signal transduction mechanisms required for normal functioning of T cells. In particular, tyrosine phosphorylation downstream of T-cell receptor activation was decreased probably due to the reduction of TCR CD3-ξ chain reexpression upon T-cell engagement. Isogenic *H. pylori* strains lacking the *arginase* gene were unable to interfere with T-cell proliferation. Furthermore, they suggest that bacteria/T cell contact is not required for this inhibitory activity. Because colonization of mice is not

compromised in arginase mutants (44), the new results confirm a role of this enzyme in the ability of *H. pylori* to survive in the gastric mucosa rather than in the establishment of the infection.

CagA and the cagPAI. *cagA* is the last gene of the *cag* pathogenicity island (*cag*PAI). The CagA protein is translocated into epithelial cells through a type IV secretion system, also within the *cag*PAI. It has been long recognized that strains carrying the *cag*PAI and specifically able to translocate CagA into host cells are associated with the development of more severe disease (ulcer, cancer versus gastritis). Once CagA is translocated into epithelial cells, it remains associated with the host membrane and becomes tyrosine phosphorylated on carboxyl-terminal repeat motifs, Glu-Pro-Ile-Tyr-Ala (EPIYA motifs), by proteins of the Src family of tyrosine kinases. Phosphorylated CagA has been reported to induce cell signaling pathways resulting in altered spreading, migration, and adhesion of epithelial cells. The Ras/MEK/ERK and SHP-2 pathways are some of the pathways reported to be activated by *cagA*-positive strains, explaining observations of increased cell proliferation during *H. pylori* infection, a hallmark for a precancerous state.

Binding of CagA to the SHP-2 protein occurs through a carboxyl-terminal domain, near the tyrosine phosphorylation EPIYA motifs, is dependent on CagA phosphorylation and stimulates SHP-2 phosphatase activity *in vitro* (45). Recently, this association has also been shown to occur in human gastric mucosa (46). Interestingly, the interaction was not detected in the gastric mucosa from patients with intestinal metaplasia or cancer. CagA protein isolated from East Asia, where gastric cancer is more prevalent, has a distinct sequence at the region corresponding to the EPIYA motifs compared with the Western CagA. The East Asian sequence confers CagA stronger binding to SHP-2 than the Western sequence (45). Recently, Azuma et al. (47) showed the association of the East Asian sequence with gastric cancer in Japan by measuring CagA strain variation in two different areas of Japan with different gastric cancer risk. In Fukui, where the risk for gastric cancer is higher, all the isolated strains harbored the East Asian sequence, whereas in Okinawa 16% of strains were of the Western sequence type.

Investigations of the molecules and pathways CagA interacts with in epithelial cells have shown association of CagA with a large number of molecules, including the COOH-terminal Src kinase (Csk; ref. 48), the adaptor molecule Grb2 (49), and ZO-1 (50). CagA has also been reported to activate the c-Met receptor pathway (51), inactivate Src kinase, and dephosphorylate cortactin (52), highlighting its role as a docking/scaffolding protein able to recruit and modify the activity of multiple signaling molecules in host cells. The advantage this situation represents for the bacterium is still not clear, but shows how infection with strains able to translocate and phosphorylate CagA can alter the physiology of gastric epithelial cells.

The presence of CagA and the *cag*PAI has also been associated with changes in epithelial cell motility and morphology *in vitro*, the so-called ''hummingbird'' phenotype. Recently, these two phenotypes were genetically separated by showing that elongation of AGS cells required CagA phosphorylation and a functional *cag*PAI, but the induction of motility was *cag*PAI independent. These results suggest that induction of motility might be influenced by bacterial factors encoded outside of the *cag*PAI (53, 54).

Whereas the majority of work with the *cag*PAI has focused on epithelial cells, recent work showed that the *cag*PAI can modulate the activity of immune cells (2, 7). The role that cagA and/or the TFSS play in immune cell modulation are open areas of investigation that will further our understanding of *H. pylori* infection.

In summary, we have learned more about the mechanisms by which CagA modify the activity of epithelial cells by serving as a scaffolding protein able to interact and modify the function of a variety of molecules involved in cell-to-cell interactions, cell motility, and proliferation. Many of these interactions need to be confirmed *in vivo*. The role of *cag*PAI products in the modulation of the immune response is an open area of research with the potential to give us more insights into the mechanisms by which *H. pylori* evades the immune response as well as a better understanding of the ability of *cag*PAI+ strains to be more pathogenic.

Conclusions

Characterization of *H. pylori* virulence factors *in vitro* has revealed many biological activities. New studies are beginning to elucidate when and where these proteins are expressed *in vivo*. This will help determine which of these activities are most relevant to the bacterium in allowing it to successfully colonize and those most relevant to the host in terms of disease progression. Interestingly, analysis of *H. pylori* gene expression during the different phases of bacterial growth highlighted the importance of stationary phase during infection. This may explain a portion of treatment failure when using antibiotics that target rapidly growing cells and should be considered when developing alternative treatment options. On the flip side, a more thorough investigation of the immune response and newly appreciated interactions between bacterial factors and cells of the immune system are giving important insights into the mechanisms by which this bacterium is able to persist for so long in the host. This long-term persistence is likely a critical factor in inducing gastric cancer progression. In this regard, the identification of dendritic cells and T regulatory cells as important immune modulators during infection may contribute to the development of effective vaccines.

The elucidation of the most critical factors for bacterial persistence and those that induce damage will be useful for identifying those individuals infected with the most virulent strains and, therefore, at greatest risk for development of severe disease. Additionally, understanding of the mechanisms of action of some of these factors may lead to the identification of early markers for disease progression. The major challenge before us is to translate these biological observations into public health measures to lower the burden of gastric cancer.

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