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REVIEW ARTICLE

Pathogenesis of *Helicobacter pylori* Infection

Trinidad Parra Cid,*,# Miryam Calvino Fernández,* Selma Benito Martínez* and Nicola L. Jones†

*Unidad de Investigación, Hospital Universitario de Guadalajara, Guadalajara, Spain, #CIBERehd (Centro de Investigación Biomédica en Red, Enfermedades Hepáticas y Digestivas), Madrid, Spain, †Division of Gastroenterology, Departments of Paediatrics and Physiology, University of Toronto, Hepatology and Nutrition, Sickkids, Toronto, Canada

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Reprint requests to: Trinidad Parra Cid, Unidad de Investigación Hospital Universitario de Guadalajara, C/Donante de Sangre s/n, 19002-Guadalajara, Spain.
E-mail: trpaci@sescam.jccm.es

Abstract

Helicobacter pylori infection and disease outcome are mediated by a complex interplay between bacterial, host, and environmental factors. Over the past year, our understanding of this complex interplay has been improved by a variety of studies focusing on both host and bacterial factors. These include studies assessing novel virulence factors as well as those most frequently associated with severity of disease outcome including *cag*A and the *cag* pathogenicity island, and the vacuolating cytotoxin. Several studies have focused on regulation of virulence factors by environmental factors. In addition, mechanisms by which bacterial virulence factors influence the host response and disease, by inducing epigenetic changes, autophagy and altered oxidative stress have also been elucidated. This review highlights key findings in the pathogenesis of *H. pylori* infection reported over the past year.

Helicobacter pylori remains an outstanding pathogen and serves as a key model system for understanding the fascinating intricacies of host–pathogen interactions in the gastrointestinal tract, as well as in infection- and inflammation-mediated cancers. This review highlights recent advances in *H. pylori* pathogenesis over the past year.

H. pylori Colonization of the Gastric Epithelium

To promote chronic infection, *H. pylori* has developed a variety of mechanisms to survive in the harsh acidic environment of the gastric mucosa. One of these is an "acid acclimation mechanism" that promotes adjustment of periplasmic pH in the acidic environment of the stomach by regulating activity of urease, UreI, and α-carbonic anhydrase. Two-component systems, which generally are composed of histidine kinases and a response regulator, are important mechanisms that allow bacteria to respond to environmental signals. Previous studies indicated that the ArsS two-component system regulated transcription of urease [1]. A recent study employing ArsS mutants extended these findings to demonstrate that ArsS also mediated trafficking of urease to the inner membrane upon acute but not prolonged acid exposure, identifying another mechanism by which the ArsS two-component system regulates acid acclimation.

Bacterial motility is also necessary for successful colonization of the gastric epithelium. Motility of *H. pylori* depends on the presence of up to 6 functional unipolar flagella. Recent studies indicate that proper assembly of flagella requires peptidoglycan-degrading enzymes that promote the correct localization and function of the flagella motor [2]. *H. pylori* regulates cell motility by responding to chemotactic cues, which then alter flagellar activity. Indeed, chemotactic (Che-) mutants have altered colonization patterns. *H. pylori* senses environmental chemical cues via four chemoreceptors: Tlp A, B, C, and D. Using isogenic chemoreceptor mutants, Rolig et al. demonstrate that TlpD is necessary for *H. pylori* to survive and grow in the infected and inflamed antrum but not elsewhere in the murine stomach [3].

After colonization, adherence to gastric epithelial cells is required to avoid shedding and increase availability of nutrients. However, adherence may also be detrimental due to more intimate interactions with the host immune response. *H. pylori* employs genetic diversification to adapt to the changing environment to promote colonization and persistent infection. *H. pylori* has

a variety of outer membrane proteins (OMPs), several of which can serve as adhesins including BabA and SabA. The 5′ and 3′ end regions of the *omp* genes (encoding OMPs) are highly conserved, which could allow for recombination, thereby switching loci and bacterial phenotype [4]. Clinical isolates obtained from pediatric subjects showed variability in the copy number and locus of the *omp* genes *sab*A and *sab*B implicating intragenomic recombination among strains [4]. In vitro studies demonstrated that *sab*A gene duplication increases SabA protein production and adherence. Using binding assays to a panel of glycosphingolipids, the structural requirements for binding of BabA to the host cell adhesin receptor were further assessed. BabA was found to bind blood group determinants on both the type 1 and type 4 core chains in these in vitro assays [5].

Bacterial Virulence Factors

Cytotoxin-Associated Gene (cag)A

Recent studies continue to expand our understanding of the potential mechanisms by which the major virulence factors *cag*A and *vac*A influence disease. Of interest, a novel model for investigating CagA pathogenesis was recently described in zebra fish [6]. This model recapitulated CagA-mediated changes previously identified in tissue culture and in animal models supporting its use to investigate pathogenic mechanisms involved in disease.

Two complementary studies provided insight into the structure and function of CagA [7,8]. Upon contact with epithelial cells, CagA is injected into the host cell via the *cag* pathogenicity island (*cag*PAI)-encoded type IV secretion system (T4SS). CagA is composed of a disordered C-terminal region that contains the EPIYA motifs and a structured N-terminal region with several conserved regions. The predicted size of CagA is larger than the channel of T4SS. Several proteins including CagL, CagY, and CagA that are present on the T4SS use beta-1 integrin as a receptor to deliver CagA into the host cell. The crystal structure of the N-terminal region of CagA identified a single-layer beta-sheet (SLB) region that acts as the functional binding domain for β1 integrin as determined by yeast two-hybrid protein-interaction screens [8]. Furthermore, CagA SLB fragments but not the RGD motif mimicking invasin blocked CagA translocation indicating that CagA uses a unique mechanism to interact with integrin to mediate injection into host cells. Upon injection, CagA is linked to the inner leaflet of the cell membrane via interactions with phosphatidylserine (PS). These studies identified a conserved basic patch in the N-terminal domain that might mediate an electrostatic interaction with PS [7]. Mutagenesis studies supported the role of this basic region in regulating the CagA–PS interaction. Thus, identification of the structure of CagA revealed important information regarding mechanisms of translocation and localization in host cells.

Once injected into the cytoplasm via the T4SS, CagA can be phosphorylated by the host and alter host cell signaling in both phosphorylation-dependent and phosphorylation-independent manner. CagA is phosphorylated on EPIYA motifs that have been classified as types A, B, C, and D on the basis of their surrounding amino acid sequences. East Asian strains have EPIYA A, B, or D, while Western strains have EPIYA A, B, or C. To define the kinetics of CagA phosphorylation during infection of gastric epithelial cells, 2 D gel electrophoresis, inhibitors and specific EPIYA motif mutants were employed [9]. This study demonstrated that CagA was phosphorylated sequentially by c-Src and then c-Abl kinases. In addition, c-Src specifically phosphorylated EPIYA C or D motifs, while c-Abl did not demonstrate specificity. The authors provided evidence that the sequential phosphorylation of EPIYA motifs is necessary for downstream signaling in host cells. A study determined that induction of heme oxygenase 1, which exhibits anti-inflammatory and antioxidant effects, reduced CagA phosphorylation during *H. pylori* infection of gastric epithelial cells in vitro [10]. Of interest, *hmox-1* expression and HO-1 protein levels were diminished in gastric epithelial cells of *cagA*+ *H. pylori*-infected patients suggesting that the bacterium may have developed a strategy to counteract *hmox-1* expression [10].

The 3′-region of the *cag*A gene in clinical isolates can vary with respect to EPIYA and CM motifs, and a variety of studies continue to elucidate the association of these variations with disease outcome in differing populations. CM is a 16 amino acid sequence responsible for CagA multimerization. In a Portuguese population, the infection with strains with higher numbers of EPIYA C was not associated with DU, but was associated with gastric precancerous lesions and increased risk of gastric cancer (GC) [11]. However, in isolates obtained from patients in three New York City hospitals, heterogeneity in EPIYA was not identified, but a great heterogeneity in CM located before and after the EPIYA C was detected [12].

CagL and the Type IV secretion system (T4SS)

The *cag*PAI encodes a T4SS, which injects CagA into host epithelial cells. A study determined that CagT (*H. pylori* 0532) was required for CagA translocation

into host cells [13]. CagL is also required for CagA translocation. CagL interacts with host cellular α5β1 integrins inducing IL-8 secretion independently of CagA translocation and NOD1 signaling [14]. All *cag*L isolates contain the RGD motif that mediates binding to integrins α5β1 and αVβ3 and activation of downstream signaling. Unlike CagA, RGD peptides derived from the CagL epitope [15] block the interaction of CagL with integrins. Previous studies suggest that CagL can decrease activity of the H^+-K^+ ATPase. However, a recent study indicates that CagL can also cause hypergastrinemia, which is a major risk factor for the development of gastric adenocarcinoma [16]. This CagL-mediated effect was independent of α5β1 but dependent on αVβ5 integrin signaling. Thus, CagL may constitute a novel target in the treatment of precancerous conditions triggered by *H. pylori*-induced hypergastrinemia [17].

Recent studies demonstrate that an additional component of the T4SS that interacts with host integrins, CagY, contains a number of repetitive amino acid motifs encoded by a large number of DNA repeats [18]. During infection in murine or primate models, recombination of these repeat regions was detected and resulted in changes in the function of CagY and the T4SS.

Vacuolating Cytotoxin (VacA)

VacA triggers intrinsic apoptosis, increases mitogen-activated protein kinases, induces autophagy (see section on autophagy) and cell death, and alters immune cell activity. Secreted VacA toxin is composed of the p33 and p55 domains that form an oligomeric structure. Structural studies of wild-type and channel-forming mutants determined that the p33 domain, which is necessary for channel-forming activity, has two globular regions that fit into the center of the oligomeric VacA complex [19]. Mutants that lack channel-forming activity do not have this organized central core region providing insight into the structure function of the toxin.

VacA from clinical isolates contains allelic variations termed the s-type, the middle region or m-type, and the intermediate region or i-type. The mosaic combination between these regions influences the levels of VacA activity and confers different risks of gastrointestinal diseases. A recent study determined that VacA i1 and i2 proteins differ in the ability to cause functional alterations in T cells in part due to altered binding. [20].

Environmental Regulation of Virulence Factors

Elucidation of the effect of environmental factors on expression of virulence factors and modulation of disease is an area of interest. Not all infected individuals develop severe complications of disease despite the presence of infection with strains expressing virulence factors suggesting an influence of both host and environmental factors on outcomes.

Loh et al. investigated the potential mechanism by which a high-salt diet could increase risk of developing gastric cancer by specifically assessing the impact of high-salt environment on bacterial protein expression [21]. Proteomic assessment of strains grown in high versus low salt identified an increase in CagA as well as in 30 other proteins upon exposure to high salt in a proportion of strains isolated from patients in Columbia. The salt-responsive CagA expression was attributed to the presence of two copies of a specific DNA motif TAATGA in the CagA promoter region, which was confirmed by mutagenesis studies. In a follow-up study using the Mongolian gerbil model, a high-salt diet was associated with increased CagA transcription and increased carcinogenesis in animals infected with the wild-type CagA+ strain [22]. Interestingly, high salt diet did not exacerbate disease in the isogenic mutant strain; however, colonization was also less efficient in comparison with the wild-type strain.

H. pylori possesses iron-scavenging mechanisms, and infection with the bacterium can induce iron-deficiency anemia both in an animal model and in humans. An interesting study demonstrated that in gerbils fed an iron-depleted diet, inflammation, dysplasia, and carcinoma were enhanced during *H. pylori* infection, which was independent of the ferric uptake regulator (*fur*) [23]. Assessment of minimally passaged isolates from iron-depleted gerbils showed increased expression of the T4SS and CagA translocation into epithelial cells in vitro in comparison with the isolates from iron-replete gerbils. In the human setting, a surrogate marker of iron deficiency, serum ferritin, was inversely associated with the severity of premalignant lesions in subjects from Colombia.

H. pylori Genetic Diversity Related to Virulence

As noted above, *H. pylori* has a great genetic diversity not only in *cag*A and *vac*A genes. Other virulence factors also harbor polymorphisms whose prevalence depends on the geographic region where the strains are isolated. A variety of studies investigating the potential for prediction of disease outcome based on the expression of allelic variants have been published in the past year with varying findings.

For example, the duodenal ulcer–promoting gene *dup*A that is predicted to form a T4SS is considered a

risk factor for DU, a protective factor for GC, and an independent risk factor for eradication failure [24]. In an Indian population, *dup*A prevalence was significantly higher among strains from patients with DU than with nonulcer dyspepsia [25]. *dup*A is highly polymorphic, and mutations that lead to truncated products are common. A study from Brazil determined that intact *dup*A was more frequently observed in strains from DU patients than in those from patients with gastritis or with GC [26].

Host Factors Implicated in pathogenesis

VacA and autophagy

Autophagy is an evolutionarily conserved process that results in the sequestration of cytosolic components within autophagosomes that then fuse with lysosomes resulting in degradation of the contents. A growing body of evidence indicates the importance of autophagy in the regulation of infection, inflammation, and carcinogenesis.

Autophagy is induced in host cells in response to VacA to mitigate the effects of the toxin. However, recent studies indicate that VacA persistence induces the formation of defective autophagosomes with attenuated ability to eliminate bacteria and potentially genotoxic material. In addition, a polymorphism in ATG16L1 results in both inefficient induction of autophagy in response to the toxin and increases susceptibility to infection in humans [27]. This study indicates that autophagy serves as a host defense response that the bacterium evades by VacA-dependent disruption of autophagosome maturation. In addition to bacterial clearance, VacA-mediated autophagy can also target CagA for removal [28]. However, in cells expressing the putative stem cell marker CD44, induction of autophagy was impaired. An additional mechanism by which *H. pylori* may subvert the autophagy pathway is by epigenetic regulation of autophagy-dependent genes. A study determined that miR30B expression was significantly upregulated during *H. pylori* infection. Furthermore, using bioinformatic tools, the autophagy regulatory genes BECN1 and ATG12 were identified as putative targets of miR30BA and shown to be downregulated by a miR30BA mimic in vitro. [29]. Thus, *H. pylori* utilizes several mechanisms to subvert the host autophagy pathway to promote its own survival. However, subversion of autophagy could result in increased expression of bacterial virulence factors as well as genotoxic material. These findings could have important implications for *H. pylori*-mediated carcinogenesis.

Oxidation and antioxidation responses related to *H. pylori* infection

H. pylori infection creates an oxidative microenvironment with release of pro-inflammatory, toxic, vasoactive substances and "reactive oxygen species (ROS)" that result in inflammation. ROS can damage major cellular constituents if the host is unable to quench the free radical overproduction [30]. Both the bacterium [31] and gastric cells respond to oxidative stress by altering the activity of their antioxidant systems. A study showed that *H. pylori* can interfere with the antioxidant response of the host. Nrf2 is a transcription factor that regulates the antioxidant response [32]. Under normal conditions, Nrf2 binds to Keap1 and undergoes proteasomal degradation [32]. However, upon oxidant stress, Nrf2 is stabilized and translocates to the nucleus to regulate transcription of antioxidant genes. *H. pylori* HspB was found to increase Keap1 expression, thereby enhancing Nrf2 degradation and impeding the host antioxidant response. HspB expression also induced increased IL-8, COX2, MMP3, and MMP7 in AGS cells suggesting a potential mechanism to enhance the inflammatory response and the destruction and remodeling of gastric tissue.

Apoptosis

Apoptosis is involved in regulating gastric cell number, and the role of this pathway in the pathogenesis of *H. pylori*-mediated gastrointestinal disorders continues to be an area of research interest. Both VacA and CagA have been implicated in regulating apoptosis. A recent study identified a *cag*PAI-mediated increase in inhibitory isoforms of p53 both in vitro and in vivo in the gerbil model [33]. Enhanced expression of these isoforms inhibited activity of p53 and p73 in association with induction of nuclear factor kappa-B (NF-κB) and indirectly promoting prosurvival signals mediated by NF-κB. It is possible that in the evolutionary adaptation process, *H. pylori* has developed mechanisms to alter cellular homeostasis without triggering cell cycle arrest or apoptosis, but increasing the risk of tumor development [34]. VacA also induces apoptosis triggered by the mitochondrial pathway mediated by binding to low-density lipoprotein receptor-related protein-1 (LRP1) and subsequently inducing autophagy prior to induction of apoptosis [35].

Epigenetic changes

A growing area of interest is the field of patho-epigenetics, which refers to epigenetic changes that occur

during infection. Epigenetic changes such as alterations in gene methylation or expression of miRNA influence the phenotypic outcome of the genome without changing the DNA code. Several recent studies have highlighted epigenetic changes mediated by *H. pylori* infection. A microarray-based assay identified differentially expressed hypermethylation of promotor regions in *H. pylori*-infected murine tissue and human gastric cancer specimens [36]. A large number of hypermethylated promotors were detected, but hypermethylation of a specific transcription factor *FOXD3* was identified both in *H. pylori*-infected murine gastric tissue and correlated with decreased survival in gastric cancer patients. Although not previously known to be a tumor suppressor, in vitro assays indicated that FOXD3 exhibited tumor suppressor function supporting a role for deregulation of FOXD3 in tumorigenesis [36]. Potential bacterial or host factors mediating hypermethylation of *FOXD3* are yet to be determined.

Additional epigenetic changes identified during infection include methylation-dependent silencing of the tumor suppressor gene E-cadherin (*E-cad*), which is identified as an early event in human gastric carcinogenesis. *H. pylori* induced *E-cad* methylation via IL-1β stimulation of the NF-κB transcriptional system leading to activation of DNA methyltransferase activity [37]. An additional mechanism by which *H. pylori* can alter E-cadherin is through cleavage by the serine protease HtrA [38]. HtrA-mediated cleavage of E-cadherin is also identified in other gram-negative pathogens and is not unique to *H. pylori*.

Micro-RNAs (miRNAs) regulate gene transcription, and many miRNAs have been implicated in tumorigenesis. In a study of cells expressing CagA, miR-26a and miR-101 expression was attenuated [39]. Using a variety of complimentary studies, the authors provided evidence for a model in which these miRs silence DNA methyltransferase. Therefore, decreased miR-26a and miR-101 expression resulted in hypermethylation of the let-7 promotor region and decreased expression of the let-7 family of miRNAs. Furthermore, the altered let-7 miRNA expression was associated with enhanced Ras expression. These findings were recapitulated in gastric tissue from CagA transgenic mice.

Conclusions

In summary, over the past year, the knowledge of factors involved in *H. pylori* disease pathogenesis continues to be elucidated and refined. As *H. pylori* is a model organism for understanding host–pathogen interactions and infection-mediated carcinogenesis, ongoing studies in this area should have broad relevance to these conditions.

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