

Helicobacter pylori

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Purpose of review

Helicobacter pylori is an important human pathogen, responsible for most peptic ulcer disease, gastritis and gastric malignancies. *H. pylori* has several unique features: it is highly adapted for gastric colonization, yet it produces clinical consequences in a small minority, its genome is known, and it is the only bacterium strongly associated with cancer. *H. pylori* is therefore of great interest to clinicians and researchers of many, often disparate, disciplines. We highlight recent advances in this fast changing field from many different areas.

Recent findings

The major contentious clinical issues relate to the synergistic gastrototoxic interactions of *H. pylori* with non-steroidal anti-inflammatory drugs, and a possible association of *H. pylori* with atherosclerotic events. Accumulating evidence implicates genetic variation in the inflammatory response to *H. pylori* in the etiology of the increased risk of gastric cancer after *H. pylori* infection. Studies of pathogenesis have been aided by increasingly sophisticated murine models. The effects in gastric epithelial cells of two of the major virulence factors (genes within the *cag* pathogenicity island and the vacuolating cytotoxin, VacA) of *H. pylori* illustrate the complex network of cellular reactions activated by *H. pylori*. The metabolism of *H. pylori* is dependent on the availability of hydrogen.

Summary

Basic science research into *H. pylori* continues to elucidate the mechanisms by which *H. pylori* infection causes disease. These findings have implications for the design of novel therapies and for improving clinical strategies to identify at-risk individuals. Many are also worthy of consideration for other epithelial-microbial interactions.

Keywords

disease associations, gastric cancer, *Helicobacter pylori*, pathogenesis

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Abbreviations

GERD	gastroesophageal reflux disease
MALT	mucosa-associated lymphoid tissue
NSAID	non-steroidal anti-inflammatory drug
PAI	pathogenicity island
SHP2	src homology 2-containing tyrosine phosphatase

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Introduction

Gastric organisms had been described over 100 years before the Gram-negative, spiral shaped, microaerophilic bacteria now known as *Helicobacter pylori* were cultured in 1982. Since then *H. pylori* has been recognized as the main cause of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [1*], and has even been implicated in the pathogenesis of some extragastric diseases, although often with scant documentation. With over 1000 English-language articles related to *H. pylori* published per annum and an entire journal devoted to this bacterium, this review will be by necessity a highly selective account of advancing knowledge regarding *H. pylori*.

Epidemiology and transmission

H. pylori is the most common chronic bacterial infection in humans. Infection is strongly correlated with socio-economic conditions, with a prevalence of over 80% in the developing world, compared with 20–50% in highly industrialized countries [1*]. *H. pylori* is highly adapted for the human stomach, where it has lived for many thousands of years, as determined by geographical associations of *H. pylori* genotypes. It is predominantly found in modern populations that derive their gene pools from ancestral populations that arose in Africa, central Asia and east Asia, with subsequent spread attributable to human migratory fluxes. An elucidation of these population patterns may aid in the development of further diagnostic tests and treatment strategies based on representative isolates [2*].

Transmission is through oral ingestion, mainly within families in early childhood. Treatment and preventive measures should therefore be most effective in children under the age of 10 years [3*]. Transmission has been documented by vomitus, saliva or feces, and possibly also through water sources in the developing world [4], but precise documentation of the major routes of transmission remains elusive. Although *H. pylori* may sometimes be eradicated by antibiotics given for other infections (especially in infants and children), infection usually persists life-long unless specific combination antibiotic therapy is taken.

Diagnostic tests

Infection with *H. pylori* can be diagnosed by endoscopic biopsy (rapid urease testing, histological examination, culture or polymerase chain reaction) or by non-invasive methods (serology, urea breath test or the detection of

H. pylori antigens in stool). The choice of an appropriate test is based on its performance and the clinical situation, as well as economic considerations. Non-invasive testing has been advocated as an alternative to endoscopy in the management of patients with dyspepsia. In a randomized trial in Scotland with 12 months' follow-up [5•], a 'test-and-treat' strategy was found to be as effective and safe as an endoscopic approach, with less patient discomfort and distress.

Disease associations

H. pylori infection is an established cause of several gastric diseases but the debate continues over whether it has any role in the etiology of disease outside the stomach.

Peptic ulcer

The two major causes of peptic ulcers are chronic *H. pylori* infection and the use of non-steroidal anti-inflammatory drugs (NSAIDs). In patients not taking NSAIDs, the eradication of *H. pylori* leads to long-term remission from chronic peptic ulcer disease. Data have been conflicting regarding interactions between *H. pylori* and NSAIDs, but in a meta-analysis, *H. pylori* infection and NSAIDs have a synergistic effect on increasing the risk of peptic ulceration and ulcer bleeding [6••]. Furthermore, Chan *et al.* [7] reported from Hong Kong that for patients with dyspepsia or a past history of peptic ulcer who require long-term NSAID treatment, screening and treatment for *H. pylori* infection significantly reduced the risks of an ulcer developing. Such evidence formed the basis of a recommendation in the Maastricht 2-2000 Consensus Report of a panel of mainly European experts that *H. pylori* should be eradicated before commencing NSAID therapy [8•].

Non-ulcer dyspepsia

Non-ulcer dyspepsia (defined as dyspepsia in the absence of a structural cause after appropriate investigation) is a common problem in clinical practice. Is *H. pylori* a culprit? Multiple well designed randomized controlled trials comparing the eradication of *H. pylori* with placebo or alternative pharmacological therapies have provided conflicting, but mainly negative, results. However, a Cochrane Collaboration systematic review concluded that treating *H. pylori* has a small but statistically significant beneficial effect in treating non-ulcer dyspeptic patients [9•]. This was also the conclusion of the Maastricht 2-2000 group, who advised the eradication of *H. pylori* in such cases [8•]. This continues to be a subject of much debate.

Gastric cancer

Up to 50% of all *H. pylori*-infected individuals will progress some way down the pre-neoplastic sequence of histological change towards gastric cancer [10], although

fewer than 2% will develop a frank malignancy. What determines whether cancer ensues? Several *H. pylori* virulence-associated genes have been found in Western populations to be associated with an increased risk of gastric cancer and pre-cancerous lesions (see virulence factors below). However, the extent of the relationship varies considerably across populations, and explains only a fraction of the association of *H. pylori* with specific diseases. A major advance in our understanding of the pathogenesis of *H. pylori* was the demonstration that the gastric immune and epithelial response to *H. pylori* are partly dependent on cytokine gene polymorphisms [11].

Subsequent studies from Japan have confirmed that IL-1 β polymorphisms do contribute to the gastric acid secretory response to *H. pylori* infection and subsequently to clinical sequelae. These outcomes range from, at one end of the spectrum, hypochlorhydria and atrophic gastritis with an increased risk of cancer to, at the other end, high acid secretion and duodenal ulcer disease [12,13].

In an important extension of this work, Figueriedo *et al.* [14••] genotyped a large population with chronic gastritis and gastric cancer for polymorphisms of the genes for both IL-1 β and its receptor, and for the *vacA* and *cagA* genotype of the infecting *H. pylori* strain. Combinatorial analysis of both bacterial and host genotypes demonstrated an enormous (up to 90-fold) difference in the risk of gastric cancer, depending on particular mixtures of *H. pylori* virulence and host genetic factors, thus demonstrating the importance of considering both *H. pylori* and host genetics in gastric cancer risk assessment.

What are the cellular and molecular mechanisms linking *H. pylori* to inflammation and gastric cancer? *In vitro*, *H. pylori* interferes with the host cellular machinery that normally limits DNA damage through mismatch repair mechanisms [15]. Chronic *H. pylori* infection is also associated with increased gastric cell turnover, probably of importance in malignant transformation. Increased gastric cell proliferation (but not increased apoptosis) may be related to the *babA2* adhesin of *H. pylori* [16]. In related work, strains of *H. pylori* carrying the *cag* pathogenicity island (PAI) were found to be anti-apoptotic, through a pathway involving nuclear factor kappa B [17•], indicating that by modulating gastric cell cycling *H. pylori* may cause the aberrant growth of neoplastic and preneoplastic gastric epithelial cells.

Mucosa-associated lymphoid tissue lymphoma

The regression of gastric low-grade MALT lymphomas after *H. pylori* therapy occurs in most cases. What should be done when gastric lymphoma persists after the successful eradication of *H. pylori*? A small case study

of seven such patients who refused further management (surgery, radiation or chemotherapy) showed that they came to no harm with a watch-and-wait strategy over approximately 3 years' follow-up [18^{*}]. Further verification by following a larger group of patients over a longer period with suitable control groups would be important for clinicians managing such patients.

In a murine model of *H. pylori*-induced MALT lymphoma that faithfully reproduces almost all of the histological features of the human malignancy, Mueller *et al.* [19^{*}] elucidated the gene expression profiles characteristic and predictive of the various histopathological stages of the disease. The study should pave the way for an improved understanding of the molecular mechanisms of MALT lymphomagenesis in humans.

Esophageal disease

Considerable controversy exists regarding the relationship between *H. pylori* and the esophagus. *H. pylori* is relatively infrequent in gastroesophageal reflux disease (GERD), and the presence of *H. pylori* improves the efficacy of proton pump inhibitors in suppressing gastric acid secretion. Because some studies suggest that the eradication of *H. pylori* may cause GERD, whereas others found *H. pylori* to be less prevalent in patients with GERD-associated distal esophageal adenocarcinoma, *H. pylori* may conceivably 'protect' against GERD and its deleterious consequences [20]. However, subsequent studies have not generally supported this hypothesis [21], and the benefits of eradicating *H. pylori* (lower risk of peptic ulcer disease and distal or non-cardia gastric cancer) probably outweigh any potentially harmful esophageal consequences.

Other associations

Helicobacter species have been implicated in the pathogenesis of many extragastric diseases, including hepatobiliary diseases, pancreatic cancer and even atherosclerosis. However, many of the studies have presented conflicting findings, and the significance of many of these associations remains uncertain. For example, a retrospective case-control analysis by Rajkumar *et al.* [22] negated a previous association of the resolution of monoclonal gammopathy of undetermined significance upon the eradication of *H. pylori*.

A nested case-control study supported the theory that the presence of serum antibodies to the CagA protein of *H. pylori* may confer an increased risk of coronary artery disease [23]. The mechanisms postulated involve anti-CagA antibodies crossreacting with exposed antigens in injured arteries [24]. CagA-positive *H. pylori* strains may also be more prevalent in patients with ischemic stroke as a result of large vessel atherosclerosis than in those with cardioembolic causes [25].

Therapy

There has been little recent advance in the therapy for *H. pylori*. One week proton pump inhibitor-based triple therapy (with clarithromycin and either amoxicillin or metronidazole) remains the treatment of choice. Quadruple therapy of a proton-pump inhibitor with bismuth subcitrate, tetracycline and metronidazole given for one week is equally efficacious and tolerable [26], and, because of the high cost of clarithromycin, may be more cost-effective and practical for the developing world, even when primary metronidazole resistance is common.

Many compounds can kill *H. pylori in vitro*, although this is often not paralleled by in-vivo activity. With this caveat in mind, it is interesting that sulforaphane (an isothiocyanate found in broccoli) kills *H. pylori in vitro*, and furthermore has a moderate chemopreventive effect on the formation of tumors in the fore-stomachs of mice induced by benzo[α]pyrene [27]. Accepting the artificiality of the systems used, this nevertheless indicates that naturally occurring compounds in the diet may be useful both to kill *H. pylori* and reduce the risk of gastric cancer.

Prophylactic oral immunization with recombinant *H. pylori* urease and 2.5 μ g *Escherichia coli* heat-labile enterotoxin as an oral adjuvant has been shown to be immunogenic in healthy volunteers, with acceptable side-effects [28]. Akhiani *et al.* [29] attempted to disentangle the inconsistencies surrounding the role of T helper types 1 and 2 cells in the development of protective immunity against *Helicobacter* infection after oral or intranasal vaccination in murine models. Their work invokes IL-12-dependent immunity mediated by T helper type 1 cells as critical for effective immunization [29].

Animal models

Animal models have been used extensively to study the immune response of *H. pylori* in order to develop suitable vaccines – a hope still unfulfilled. The immune response to *H. pylori* in mice appears to be very similar to that in humans at a proteomic level [30]; therefore, this approach may be useful for defining immunodominant targets.

Animals infected with *H. pylori* infection continue to provide important information regarding pathogenesis. The ready availability of reagents for murine tissue and the ability to manipulate mice genetically, together with the availability of mouse-adapted strains of *H. pylori* (such as SS1) [31], have led to the dominance of the mouse model. Very young (5–6-day-old) mice are particularly susceptible to experimental infection, and can easily be infected with non-adapted *H. pylori* strains [32^{*}]. These suckling mice may therefore represent an ideal model for investigating early events in *H. pylori*

colonization, including competition between different *H. pylori* strains for the gastric niche, for finding *H. pylori* genes essential for early colonization, and in evaluating the immune response to primary infection.

The only rodent model that reproducibly develops gastric cancer after *H. pylori* infection is the INS-GAS mouse, an inbred FVB strain, with the human gastrin transgene under the control of the insulin promoter. Such mice develop moderate hypergastrinemia and then gastric cancer, which is accelerated by infection with *Helicobacter* species including *H. pylori*. For reasons that are currently unclear, cancer only develops in the males [33•]. This intriguing observation is consistent with the increased incidence of gastric cancer found in men.

H. pylori virulence factors

H. pylori is an extremely heterogeneous species. The recent sequencing of two *H. pylori* plasmids has found chromosomally encoded genes and elements susceptible to recombination and transposition that could contribute to this heterogeneity [34]. Research into specific *H. pylori* virulence factors continues intensively. OipA is an outer membrane protein whose function is dependent on slipped strand repair of CT dinucleotide repeats in the 5' region of the gene – a functional protein resulting from six or nine such repeats. In a large analysis of *H. pylori* strains from the United States and Colombia, South America, the OipA status was more closely associated with disease (duodenal ulcer or gastric cancer) than was either the presence of the *cag* pathogenicity island, the *baba2* gene or *vacA* status [35•].

Another newly identified candidate virulence gene, *sabA*, encodes an adhesin that binds to the Lewis X antigen on inflamed gastric epithelial cells. Interestingly, sialylated Lewis X is known to be upregulated in chronic inflammation and cancer. An exhaustive series of binding studies has now shown that *H. pylori* upregulates sialyl-Lewis X expression in humans and in a monkey model, providing a binding site for SabA [36•]. Furthermore, the expression of SabA by *H. pylori* is linked to possession of the *cag* PAI.

VacA

The *vacA* gene of *H. pylori* encodes a multimeric exotoxin producing endosomal vacuoles in epithelial cells. *H. pylori* strains expressing the high activity forms of VacA are associated with an increased risk of ulcer disease. This had been thought to be due to the ability of VacA to bind to gastric epithelial cell receptors and, after internalization, to form endocytic vacuoles and cell death. However, mice deficient in protein tyrosine phosphatase receptor type Z incorporate VacA and demonstrate vacuolization but do not develop ulcers [37•]. VacA may therefore act as a ligand for protein

tyrosine phosphatase receptor type Z, although other groups have proposed other proteins and structures including lipid rafts [38] to be the elusive VacA receptor.

cag Pathogenicity island

Most *H. pylori* strains contain the *cag* PAI, a region with a different GC content to the rest of the *H. pylori* genome, which suggests that it was originally acquired by horizontal transmission from another species. Carriage of the *cag* PAI is associated with the induction of a more severe inflammatory response and, in Western populations, with disease states (peptic ulcer disease and gastric cancer). The relationship between *cag* carriage and disease is much less clear-cut in the developing world, where almost all strains are *cag*-positive [39].

The *cag* PAI comprises approximately 30 open reading frames, including several that form a type IV secretory apparatus capable of transferring bacterial products directly into gastric epithelial cells. Subsequently, the modulation of epithelial cell signal transduction pathways results in the activation of inflammatory cascades and other events of potential importance in pathogenesis. One bacterial protein transferred to epithelial cells is the immunodominant CagA protein, which is then tyrosine phosphorylated by intracellular *src* homology 2-containing tyrosine phosphatase (SHP2). This anchors SHP2 to the plasma membrane and activates downstream pathways [40•]. Further work from this same group has demonstrated that the CagA proteins of strains from east Asia (where gastric cancer is more common) possess unique tyrosine phosphorylation sequences within CagA that confer stronger binding to SHP2 than CagA from Western *H. pylori* isolates [41•].

The tyrosine phosphorylation of CagA by Src family tyrosine kinases is itself downregulated by phosphorylated CagA [42], leading through cortactin dephosphorylation to actin rearrangement and crosslinking, resulting in a 'spreading' or 'hummingbird' morphology in the AGS gastric epithelial cell line. This change is believed (but not proved) to be important for neoplastic transformation. The transfection of tyrosine phosphorylated CagA inhibits Src activation [42], but the pathway may be indirect, through stimulating C-terminal Src kinase activity and inactivating Src family protein tyrosine kinases [43]. Furthermore, the ectopic overexpression of CagA induces apoptosis through the activation of SHP2 [43]. CagA may therefore stimulate both oncogenic and apoptotic pathways simultaneously.

CagA may also activate signal transduction without being tyrosine phosphorylated. CagA derivatives resistant to tyrosine phosphorylation can also induce the hummingbird phenotype and increase cell proliferation by interacting with Grb2 and the Ras/MEK/ERK MAP kinase

pathway [44]. Another phenomenon dependent on CagA, but seemingly independent of tyrosine phosphorylation, is the transcription of the serum response element – a sequence common to the promoter regions of the intermediate early genes *c-fos* and *egr-1* [45].

Many genes within the *cag* PAI are involved in the structural assembly of the type IV secretion system, but the function of others remains obscure. A strategy using high-density microarray transcriptional profiling and hierarchical clustering to examine the responses of gastric epithelial cells to isogenic mutant strains, specifically lacking each of the *cag* genes, may be helpful to dissect the functions of individual *cag* PAI genes further [46•].

Growth and metabolism

H. pylori occupies a unique niche; it is highly adapted to survive over decades in a microaerophilic, extremely acidic environment. The urease of *H. pylori* is essential for colonization and survival at extremely low pH. To ensure cytoplasmic homeostasis during the large pH changes that occur during feeding, for example, its urease activity must be tightly regulated, but details of the responsible mechanisms remain controversial [47]. In addition to its role in regulating intrabacterial pH, urease may have other functions, such as inducing apoptosis [48] and in stimulating the production of inducible nitric oxide synthase from macrophages [49].

H. pylori can use molecular hydrogen as an energy source [50•]; thus, its growth may depend to some extent on the hydrogen excreted by colonic and intestinal bacteria (potentially reaching the stomach through diffusion upwards or via the expired breath). *H. pylori* strains lacking functional endonuclease III (an enzyme essential for the repair of oxidized pyrimidine bases) colonize the stomach very poorly and lose out in competition with wild-type strains. The selection advantage provided by functional endonuclease III is probably caused by resistance against the oxidative stress generated in the inflammatory response to *H. pylori* [51].

Utilizing the available genomic information regarding *H. pylori* strain 26695, an in-silico metabolic model has been constructed [52]. This may be used to develop strategies to define the essential amino acids and other constituents that could perhaps be modulated as part of the treatment to eradicate *H. pylori* and to identify essential genes for targeting. Is the gastric acid-secreting peptide gastrin critical for *H. pylori* growth? *H. pylori* stimulates gastrin release from antral neuroendocrine cells and, following up from previous work, a 13-amino acid sequence within human gastrin promotes the growth of *H. pylori*. *H. pylori* may thus serve to stimulate its own growth, albeit very indirectly [53•].

Transcriptome

The pattern of genes expressed by *H. pylori* during attachment to the gastric epithelium is quite different from that expressed during laboratory passage. Using the method of complimentary DNA analysis termed SCOTS (selective capture of transcribed sequences), genes selectively transcribed on attachment to the gastric mucosa (human or gerbil) comprised approximately 70% of all *H. pylori*'s known open reading frames [54•]. Some of these were expressed only on binding to gastric tissue, possibly representing genes essential for virulence and gastric adaptation.

Part of the pro-apoptotic activity of *H. pylori* derives from secreted proteins, including VacA [55] and a recently-identified outer membrane protein with gammaglutamyl transpeptidase activity [56]. However, *H. pylori* secretes multiple other proteins into its surroundings. The analysis of the 'secretome' had previously been difficult, owing to the tendency for *H. pylori* to spontaneously lyse in culture, releasing intracellular proteins non-physiologically. Recently, two groups carefully analyzed the secreted proteins of *H. pylori*, after correcting for intracellular lysis [57,58]. Among the secreted proteins emerged VacA, sequences of the same gammaglutamyl transpeptidase reported to have apoptosis-inducing activity [56], thioredoxins and other proteins with oxidoreductase activity capable of modifying disulfide bonds. Further analysis of the function of many of these proteins is awaited with interest.

Conclusion

The investigation of *H. pylori* continues to provide new insights into the pathogenesis of diseases not formerly viewed as infectious in origin. Clinically, there is considerable debate but little new information regarding *H. pylori* and NSAID-induced gastric damage, its role in non-ulcer dyspepsia and its relationship with esophageal disease. The availability of genomic sequences for *Homo sapiens* and *H. pylori* has stimulated basic research into dissecting the involvement of *H. pylori* with gastric malignancies at a molecular level. This work is providing tantalizing new directions for translational research, with a high probability of eventual clinical application.

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