HELICOBACTER PYLORI AND GASTROINTESTINAL TRACT ADENOCARCINOMAS

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Although gastric adenocarcinoma is associated with the presence of *Helicobacter pylori* in the stomach, only a small fraction of colonized individuals develop this common malignancy. *H. pylori* strain and host genotypes probably influence the risk of carcinogenesis by differentially affecting host inflammatory responses and epithelial-cell physiology. Understanding the host–microbial interactions that lead to neoplasia will improve cancer-targeted therapeutics and diagnostics, and provide mechanistic insights into other malignancies that arise within the context of microbially initiated inflammatory states.

GASTROESOPHAGEAL REFLUX DISEASE
(GERD). A condition in which gastric contents are refluxed into the oesophagus, characterized by the symptom of heartburn.

GASTROENTERITIS
Acute gastrointestinal infection caused by bacterial or viral agents. It is characterized by nausea, vomiting and diarrhoea.

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Gastric adenocarcinoma is the second leading cause of cancer-related death in the world[1]. Epidemiological and interventional studies in humans, as well as experiments in rodents, have associated *Helicobacter pylori* — a member of a large family of related bacteria that colonize the mammalian stomach — with peptic ulcers, non-Hodgkin's lymphoma of the stomach, gastric atrophy and distal gastric adenocarcinoma[2–10]. However, only a small percentage (probably less than 3%) of individuals that carry *H. pylori* ever develop neoplasias related to its presence, indicating that other factors are involved. Such observations, along with recent evidence that certain *H. pylori* strains might reduce the risk of GASTROESOPHAGEAL REFLUX DISEASE (GERD) and its complications (for example, oesophageal adenocarcinoma)[11,12], underscore the importance of understanding the biological interactions of these organisms with their host.

H. pylori epidemiology

H. pylori is present in the stomachs of at least half of the world's population. It is usually acquired in childhood, and when left untreated generally persists for the host's lifetime[13]. Once established, *H. pylori* has no significant bacterial competitors and — except for transient bacteria — the stomach is essentially a monoculture of *H. pylori*. Although people in all geographical zones carry the bacteria, the prevalence of *H. pylori* is higher in developing countries than in developed countries[13,14]. In the United States, *H. pylori* is present in 10–15% of children who are less than 12 years old, compared with 50–60% of people greater than 60 years old[13–15]. The rate of acquisition of new *H. pylori* infections among adults in developed countries is less than 1% per year[14], and most American carriers probably acquired *H. pylori* during childhood. Over the past half-century, however, progressively fewer children have been shown to carry *H. pylori* — this decrease has been accelerated by the widespread use of antibiotics. Risk factors for *H. pylori* acquisition include low socioeconomic status, household crowding, country of origin and ethnicity[13,16]. Colonization is related to intrafamilial clustering, but not to the presence of non-primate reservoirs, indicating that transmission of *H. pylori* from person to person occurs[13]. Induction of regurgitation and catharsis increased the chance of obtaining a positive *H. pylori* culture from vomitus and diarrhoeal specimens[17], which indicates that *H. pylori* transmission might be associated with childhood episodes of GASTROENTERITIS.

H. pylori and gastric cancer

Two histologically distinct variants of gastric adenocarcinoma have been described, each having

Summary

- Gastric adenocarcinoma is the second leading cause of cancer-related deaths in the world, and has been associated with the presence of *Helicobacter pylori* in the stomach.
- Gastric cancer involves a transition from normal mucosa to gastritis, which then leads eventually to adenocarcinoma. The ability of *H. pylori* to induce superficial gastritis indicates that it is involved in the initiation and promotion of gastric neoplasia. Many clinical and animal studies support this idea.
- *H. pylori* populations are extremely diverse, due to point mutations, substitutions, insertions and/or deletions in their genomes. Cancer risk is believed to be related to *H. pylori* strain differences.
- There are also a number of human polymorphisms associated with gastric cancer. Most of these occur within immune-response genes.
- *H. pylori* have a number of direct effects on host epithelial tissues that could affect tumorigenesis, including induction of proliferation, the inflammatory response and apoptosis.
- So, host and pathogen are likely to be linked in a dynamic equilibrium, in which the host responses to bacterial colonization affect the growth of certain bacterial strains, and strain phenotype affects the nature of the host response.
- Remarkably, the presence of *H. pylori* reduces the risk of developing other types of cancer, such as oesophageal adenocarcinoma. The same biological effects of *H. pylori* that predispose people to gastric cancer are likely to protect them from oesophageal cancer.

different epidemiological and pathophysiological features. Intestinal-type gastric adenocarcinoma usually occurs at a late age, predominates in men and progresses through a relatively well-defined series of histological steps[18]. Diffuse-type gastric adenocarcinoma more commonly affects younger people, affects men and women equally and consists of individually infiltrating neoplastic cells that do not form glandular structures and are not associated with intestinal metaplasia[18]. Although *H. pylori* significantly increases the risk of developing both subtypes of gastric adenocarcinoma, the mechanisms underpinning the development of intestinal-type cancer are more well-characterized; therefore, the remainder of this review will focus predominantly on the relationships between *H. pylori* and intestinal-type gastric adenocarcinoma.

The chain of events that occurs during development of intestinal-type gastric cancer involves a transition from normal mucosa to chronic superficial GASTRITIS, which then leads to ATROPHIC GASTRITIS and INTESTINAL METAPLASIA, and finally to DYSPLASIA and ADENOCARCINOMA[1] (FIG. 1). The risk of developing gastric cancer increases exponentially as the extent of atrophic gastritis and intestinal metaplasia increases, and patients with severe multifocal atrophic gastritis have over a 90-fold greater risk of developing adenocarcinoma than those with normal mucosa[18]. In contrast to the apparent 'orderly' sequence of genetic mutations that accumulate during colorectal carcinogenesis, no mutational events are consistently associated with intermediate steps in the progression to intestinal-type gastric adenocarcinoma (FIG. 1)[19–21]. The ability of *H. pylori* to induce superficial gastritis[22], however, indicates that this organism — or the host inflammatory response to it — could be important in the initiation and promotion of gastric neoplasia.

Epidemiological studies indicate that *H. pylori* colonization increases the risk of developing distal (non-cardia) gastric cancer (FIG. 2). The progressive decline in *H. pylori* acquisition during the last century by people living in developed countries has been mirrored by a decreasing incidence of these gastric cancers[23,24]. Several case-controlled studies have shown that *H. pylori* seropositivity is associated with a significantly increased risk of gastric cancer (2.1–16.7-fold greater than in seronegative persons)[5–10,25–30]. In developed countries, *H. pylori* probably increases the risk of developing gastric cancer by sixfold[31]. The actual risk of gastric cancer that is attributable to *H. pylori* might be even higher, because *H. pylori* colonization diminishes in the presence of premalignant lesions, such as gastric atrophy or intestinal metaplasia, making it difficult to detect in all patients. Importantly, prospective studies have shown that the longer the time interval between *H. pylori* detection and gastric cancer diagnosis, the higher the risk of developing cancer[31].

Results from several studies, reporting that antimicrobial treatment alters gastric carcinogenesis, also implicate *H. pylori* in the progression to neoplasia. A randomized controlled chemoprevention trial showed that antimicrobial therapy directed against *H. pylori* increased the regression rate of gastric atrophy and intestinal metaplasia, compared with patients receiving placebo[32]. In Japanese patients with early gastric cancer, therapy to eliminate *H. pylori* resulted in a significantly lower rate of gastric cancer

GASTRITIS
Inflammation within the gastric mucosa. Gastritis induced by *H. pylori* involves polymorphonuclear cells, lymphocytes (T and B cells), macrophages and plasma cells.

ATROPHIC GASTRITIS
An intermediate histological step in the progression to intestinal-type gastric adenocarcinoma, characterized by variable gland loss and encroachment of inflammatory cells into the glandular zones.

INTESTINAL METAPLASIA
A premalignant histological lesion in the progression to intestinal-type gastric adenocarcinoma, in which normal gastric mucosa is replaced by intestinal-type epithelial cells.

DYSPLASIA
Neoplasia that involves lining epithelial cells that have not breached the basement membrane (which separates epithelial cells from the underlying lamina propria), and the capacity to metastasize is therefore absent.

ADENOCARCINOMA
Fully transformed malignant tissue arising from glandular epithelium.

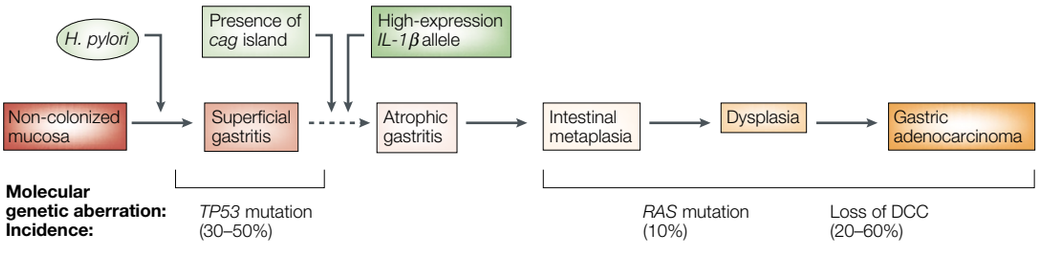

Figure 1 | **Progression to intestinal-type gastric adenocarcinoma.** *Helicobacter pylori* colonization usually occurs during childhood and, over a period of days to weeks, leads to superficial gastritis. The presence of host *TP53* mutations, host polymorphisms that promote high expression levels of the cytokine interleukin (IL)-1β, and the *cag* island within infecting *H. pylori* isolates all contribute to the development of atrophic gastritis, intestinal metaplasia, dysplasia and, eventually, gastric adenocarcinoma over the course of many years. Additional mutations in oncogenes that encode RAS or deleted in colorectal cancer (DCC) might also contribute to intestinal-type gastric carcinogenesis.

HORIZONTALLY ACQUIRED
Transfer of DNA from one bacterial species to another.

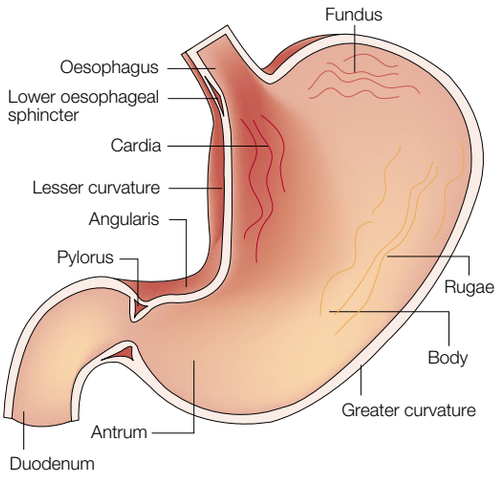

Figure 2 | **Gastric anatomy.** Anatomical arrangement of the distal oesophagus, stomach and proximal duodenum. *Helicobacter pylori*-induced inflammation can occur at any site within the stomach. However, most intestinal-type and diffuse gastric adenocarcinomas associated with *H. pylori* occur in the gastric antrum, body, or (less likely) fundus. Oesophageal adenocarcinomas — which are a complication of gastroesophageal reflux disease and Barrett's oesophagus, and are inversely related to the presence of *H. pylori* — occur in the distal oesophagus, just above and/or involving the lower oesophageal sphincter.

recurrence and reduced progression of atrophic gastritis[33]. In a recent long-term prospective study, *H. pylori* eradication prevented, or at least delayed, the development of gastric adenocarcinoma during a mean follow-up period of 4.8 years[34].

H. pylori also induces gastric cancer in rodent models. Following experimental challenge with *H. pylori*, Mongolian gerbils consistently develop pan-gastritis[35], which leads, over the course of 1–2 years, to gastric atrophy, intestinal metaplasia and intestinal-type gastric adenocarcinoma in up to one-third of animals[36,37]. The pattern of gastric cancer development in these animals parallels that of humans (FIG. 1), making this a good model of gastric carcinogenesis. Malignancy can be induced by *H. pylori* colonization alone, without the exogenous administration of co-carcinogens. Accordingly, the World Health Organization has classified *H. pylori* as a class I carcinogen of gastric cancer[38].

Table 1 | ***H. pylori* genes associated with gastric cancer**

Genetic locus		
***cag* island**	***vacA***	***babA2***
Conservation between strains		
60–70% Western strains 95–100% Asian strains	Always present, alleles vary	~85%
Function		
Forms scaffold apparatus that allows bacterial protein(s) to enter host epithelial cells	?	Bacterial adhesion to cell surface
Epidemiological disease association		
Peptic ulcer disease, gastric cancer	Peptic ulcer disease, gastric cancer	Peptic ulcer disease, gastric cancer
Genotype associated with disease		
cagA$^+$	*vacA*s1m1	*babA2*$^+$

Strain variation and disease risk

If *H. pylori* is the strongest identified risk factor for the development of distal gastric cancer, why do most carriers never develop this malignancy? Cancer risk is believed to be related to *H. pylori* strain differences, inflammatory responses governed by host genetics, and specific interactions between host and microbial determinants. *H. pylori* populations are extremely diverse[39], owing to point mutations, substitutions, insertions and/or deletions in their genomes[40]. A single host can carry several *H. pylori* strains, and isolates within an individual can change over time as endogenous mutations, chromosomal rearrangements or recombination between strains occurs[40,41]. Although this extraordinary diversity has made it difficult to search for bacterial factors that are associated with malignancy, several genetic loci have been identified, including the *cag* pathogenicity island, the *vacA* gene and the *babA2* gene (TABLE 1). These markers seem to be interdependent, and are not absolutes, but reflect degree of risk[42,43].

The cag island. The most important distinguishing factor of *H. pylori* strains is presence of the *cag* island, a HORIZONTALLY ACQUIRED locus of approximately 40 kb that contains 31 genes[44,45]. Several *cag* island genes have homology to genes that encode type IV secretion system proteins, which export proteins from bacterial cells. The terminal gene in the island, *cagA*, is commonly used as a marker for the entire *cag* locus. Following *H. pylori* adherence to epithelial cells, the secretion system translocates the CagA protein from *H. pylori* into the epithelial cell, where it undergoes tyrosine phosphorylation — a process that is associated with dephosphorylation of host-cell proteins[46–48] and host-cell morphological changes[49]. The phosphorylated form of CagA might therefore function as a phosphatase that regulates organization of the actin cytoskeleton.

Compared with *cagA*$^-$ strains, *H. pylori cagA*$^+$ strains significantly increase the risk of developing severe gastritis, atrophic gastritis, peptic ulcer disease and distal gastric cancer[50–56] (FIG. 1). *In vitro* studies have shown that several genes within the *cag* island (*cagE* (*picB*), *cagG*, *cagH*, *cagI*, *cagL*, *cagM*, but not *cagA*) are required for release of pro-inflammatory cytokines induced by *H. pylori*, such as interleukin (IL)-8, from gastric epithelial cells[57–59]. Inactivation of these same genes also results in decreased activation of the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signal-transduction cascades, which regulate pro-inflammatory cytokine production[59–63]. These *in vitro* observations mirror *in vivo* events, as *cag*$^+$ strains are associated with increased mucosal expression of IL-8 and inflammation in human gastric tissue[52,64]. Furthermore, loss of *cagE* or the entire *cag* locus profoundly attenuates the severity of

Table 2 | **Human genetic polymorphisms that influence development of distal gastric cancer**

Gene	Function of gene product	Polymorphism associated with enhanced risk	Relative risk of gastric cancer; odds ratio (95% confidence intervals)	Comment
IL-1β	Induces expression of inflammatory cytokines; potently inhibits acid secretion from parietal cells	–31 C/C –511 T/T	2.5 (1.6–3.8) 2.6 (1.7–3.9)	Cancer risk increased compared with individuals positive for *H. pylori* with 'low-expression' alleles[87]; also increases risk for atrophic gastritis
IL-1Rβ	Receptor for IL-1β	Penta-allelic 86-bp tandem repeat in intron 2	2.9 (1.9–4.4)	REF. 87
TNF-α	Activates intracellular signalling pathways related to inflammation and apoptosis; inhibits acid secretion from parietal cells	–308 A/A	1.9 (1.2–2.8)	REF. 91
IL-10	Inhibits production of pro-inflammatory cytokines	–592 ATA/ATA –819 ATA/ATA –1082 ATA/ATA	3.4* (1.4–8.1)	'Low-expression' polymorphism associated with increased cancer risk[93]

*Represents combined relative risks for *IL-10* ATA genotype compared with GCC genotype. IL, interleukin; TNF, tumour necrosis factor.

gastritis and development of atrophy in Mongolian gerbils infected with *H. pylori*[65,66].

The vacA gene. Another gene that is associated with carcinogenesis induced by *H. pylori* is *vacA*. *vacA* encodes a secreted protein that induces vacuole formation in eukaryotic cells and stimulates epithelial-cell apoptosis[67–70]. Approximately 50% of *H. pylori* strains express the VacA protein[71], and expression is correlated with expression of *cagA*[72,73]. However, *vacA* and *cagA* map to separate loci on the *H. pylori* chromosome, and an inactivating *cagA* mutation does not affect VacA production[74]. *H. pylori* VacA-secreting strains are more common among patients with distal gastric cancer than among patients with gastritis alone[75]. Unlike the *cag* island, all *H. pylori* strains possess the *vacA* gene[76], and expression differences between strains are due to sequence variations in *vacA*[76]. Regions of major sequence diversity are localized to both the *vacA* secretion-signal sequence (allele types s1a, s1b, s1c or s2) and the mid-region (allele types m1 or m2)[76–78]. Strains possessing the m1 allele are associated with enhanced gastric epithelial-cell injury[79,80] and distal gastric cancer[43,78] compared with *vacA* m2 strains.

The babA2 gene. BabA, encoded by the strain-specific gene *babA2*, is a member of a family of highly conserved outer-membrane proteins, and binds the Lewis^b (Le^b) histo-blood-group antigen on gastric epithelial cells[43,81]. *H. pylori* strains that possess the *babA2* gene are associated with an increased incidence of gastric adenocarcinoma[43]. The presence of *babA2* is correlated with the presence of *cagA* and *vacA* s1; strains that possess all three of these genes carry the highest risk of gastric cancer[43]. Following challenge with *babA2*$^+$ *H. pylori* strains, transgenic Le^b-expressing mice are more likely to develop severe gastritis, atrophy and anti-PARIETAL CELL antibodies (reflecting autoimmune tissue destruction) than their wild-type littermates[82]. BabA-expressing strains also adhere more tightly to epithelial cells, which might promote pathogenesis.

Host polymorphisms and gastric cancer

Just as certain *H. pylori* genetic elements are associated with gastric cancer, several human polymorphisms are also associated with the disease. Most of these occur within immune response genes. *H. pylori* induces a T-helper (T_H)1-type cellular immune response in humans, whereas a closely related bacteria, *Helicobacter felis*, induces the same reaction in mice[83,84]. Experimental induction of a T_H2-type immune response attenuates the gastritis and atrophy response that is observed in mice infected with *H. felis*[85], indicating that mucosal inflammation might promote tumorigenesis.

Expression levels of the T_H1 cytokine IL-1β are increased within the gastric mucosa of *H. pylori*$^+$ individuals[86], and several polymorphisms have been identified in the *IL-1β* gene promoter region that affect protein expression. Individuals that are colonized by *H. pylori*, and that possess promoter-region polymorphisms associated with higher-than-average expression levels of IL-1β, are at significantly increased risk of developing HYPOCHLORHYDRIA, gastric atrophy and distal gastric adenocarcinoma than individuals with polymorphisms linked to lower expression levels of IL-1β (REF. 87; FIG. 1; TABLE 2).

Experiments in rodent models have led to similar findings. In Mongolian gerbils infected with *H. pylori*, gastric mucosal IL-1β levels increase 6–12 weeks after bacterial infection, accompanied by a reciprocal decrease in gastric-acid production[88]. Administration of recombinant IL-1 receptor antagonist to gerbils infected

PARIETAL CELL
Highly specialized cell located in gastric glands within the gastric body and fundus that is responsible for acid secretion.

T_H1 RESPONSE
A T-helper-1 cell-mediated immune response is mediated by pro-inflammatory cytokines such as IFN-γ, IL-1β and TNF-α. It promotes cellular immune responses against intracellular infections and malignancy.

T_H2 RESPONSE
A T-helper-2 response involves production of cytokines, such as IL-4, which stimulate antibody production. T_H2 cytokines promote secretory immune responses of mucosal surfaces to extracellular pathogens and allergic reactions.

HYPOCHLORHYDRIA
Decreased secretion of acid by the stomach, often as a result of atrophic gastritis or use of acid-suppressive medications, such as proton-pump inhibitors.

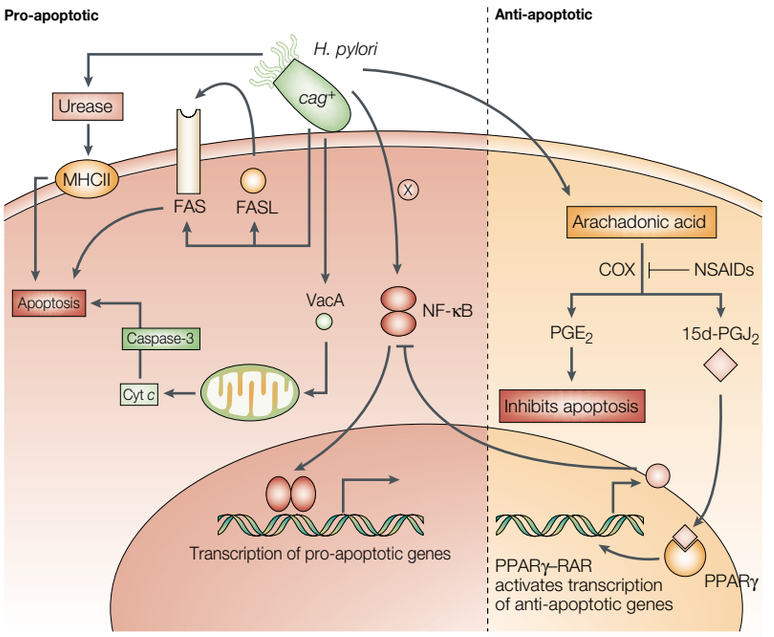

Figure 3 | ***H. pylori cag*⁺ strains can induce or prevent gastric epithelial-cell apoptosis.** *H. pylori* can regulate gastric epithelial apoptosis through several mechanisms. Following adherence, signalling by the *cag* secretion system (but not CagA *per se*) leads to activation of an unknown factor(s) X that leads to activation of nuclear factor-κB (NF-κB). NF-κB translocates to the nucleus to activate transcription of pro-apoptotic genes. *H. pylori* can also induce apoptosis by stimulating expression of FAS and its ligand (FASL). The *H. pylori* protein urease can induce apoptosis by binding to class II major histocompatability complex (MHC) molecules. The *H. pylori vacA* gene product causes mitochondrial release of cytochrome *c* (cyt *c*), which leads to activation of caspase-3 and apoptosis. *H. pylori* also activates pathways that downregulate apoptosis. *H. pylori* binding to the epithelial-cell surface generates arachadonic acid, which is metabolized to prostaglandin E_2 (PGE_2) and prostaglandin 15-deoxyΔ12,14-J_2 (15d-PGJ_2) by cyclooxygenase (COX) enzymes. These enzymes are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs). 15d-PGJ_2 is an endogenous ligand of peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear hormone receptor that heterodimerizes with the retinoid (RAR) family of nuclear receptors to activate transcription of target genes. These gene products inhibit NF-κB activation, however, preventing apoptosis. The COX-generated metabolite PGE_2 also attenuates apoptosis. So, *H. pylori* has the capacity to stimulate and inhibit gastric epithelial-cell apoptosis, which might influence the risk of gastric carcinogenesis.

with *H. pylori* normalizes acid levels[88], indicating that IL-1β is an important determinant of acid secretion within inflamed mucosa. As IL-1β is the most powerful inhibitor of acid secretion known, is profoundly pro-inflammatory, and is upregulated by *H. pylori*, this cytokine probably has a pivotal role in initiating the progression towards gastric adenocarcinoma[89].

Expression of tumour necrosis factor (TNF)-α — another T_H1 (pro-inflammatory and acid-suppressive) cytokine — is also increased within mucosa[90] colonized by *H. pylori*. Polymorphisms in the gene that encode it have been associated with an increased risk of gastric cancer and its precursors[91] (TABLE 2). Two different *H. pylori* proteins (urease B and membrane protein 1) have recently been shown to induce TNF-α expression and transformation in cells that constitutively overexpress the oncogenic protein RAS, indicating that enhanced levels of mucosal TNF-α produced by *H. pylori* in genetically susceptible individuals might contribute to carcinogenesis by interacting with activating *RAS* mutations[92]. Conversely, polymorphisms that reduce expression of the anti-inflammatory cytokine IL-10 have been associated with an enhanced risk of distal gastric cancer[93] (TABLE 2).

Some studies indicate that particular MAJOR HISTOCOMPATABILITY COMPLEX (MHC) genotypes also influence gastric carcinogenesis that is induced by *H. pylori*. Cells that express class II MHC molecules regulate the immune response by binding antigen, processing it and presenting it to $CD4^+$ T cells. Class II MHC molecules are expressed on gastric epithelial-cell surfaces and are upregulated in the presence of *H. pylori*[94], indicating that the host MHC class II haplotype might partially determine the epithelial-cell response to the pathogen. For example, host possession of the MHC *DQA1*0102* allele was reported to increase the risk of atrophic gastritis and intestinal-type gastric adenocarcinoma associated with *H. pylori*[95] . Other studies have shown that inactivating mutations in *CDH1*, the gene that encodes E-cadherin, are associated with familial diffuse-type gastric cancer[96]; however, a relationship between E-cadherin and *H. pylori* has not been established.

Biological effects of *H. pylori*

Proliferation. What effect does *H. pylori* have on the gastric epithelium that leads to cellular transformation? One effect is interference with epithelial-cell proliferation. Co-culture of *H. pylori* with epithelial cells has been shown to reduce expression of the cell-cycle regulatory protein p27, which leads to epithelial-cell G_1 arrest[97,98]. Cell-cycle arrest might be induced by DNA damage to epithelial cells. When *H. pylori* is incubated with epithelial cells, direct damage to host-cell DNA occurs through the synthesis of reactive oxygen species, as reflected by the formation of DNA adducts[99,100].

The host response to *H. pylori* can also induce epithelial-cell proliferation. These pathogens have been reported to induce hypergastrinaemia[101] — the increased production of the hormone gastrin by mucosal G-CELLS. Gastrin stimulates gastric epithelial-cell proliferation *in vitro* by activating its receptor, CCK-β[102]. Gastrin-deficient and gastrin-receptor-deficient mice develop altered glandular architecture[103] as a result of altered epithelial-cell proliferation. The ability to stimulate gastrin production might be an important aspect of tumorigenesis induced by *H. pylori*. In transgenic mice that overexpress gastrin, gastric adenocarcinomas developed in 75% of animals over 20 months old[104]. When these transgenic mice were infected with *H. felis*, 85% developed gastric carcinomas by eight months of age[104]. High gastrin levels might therefore synergize with other consequences of *H. pylori* colonization to promote gastric cancer.

Inflammation. *H. pylori* also activates pro-inflammatory cyclooxygenase (COX) enzymes (FIG. 3). The COX enzymes (COX-1 or COX-2) catalyse key steps in the formation of inflammatory prostaglandins[105]. COX-1 is expressed constitutively, whereas COX-2 is induced by cytokines such as TNF-α, interferon (IFN)-γ, and IL-1 (REF. 105). COX-2 expression is increased in

MAJOR HISTOCOMPATIBILITY COMPLEX
(MHC). Locus of genes that encode products essential to immune function. Class I and Class II MHC genes encode proteins that are involved in antigen presentation to T cells.

G-CELLS
Endocrine cells located in the gastric antrum that secrete the hormone gastrin.

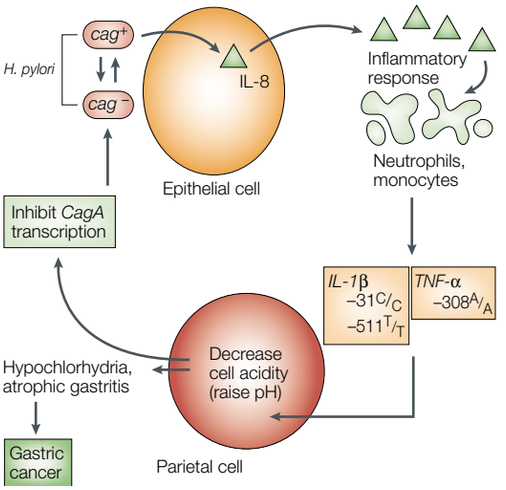

Figure 4 | **Equilibrium of interactions between *H. pylori* and its host.** *H. pylori* cells signal the host and the host signals the bacterial population. *H. pylori cag*$^+$ strains promote production of the cytokine interleukin (IL)-8 by host cells, which amplifies the inflammatory response by recruiting neutrophils and monocytes. These cells release pro-inflammatory cytokines such as IL-1β and tumour necrosis factor (TNF)-α, which reduce acid production by parietal cells, causing a decrease in gastric acidity and hypochlorhydria. This increases the risk of atrophic gastritis, a pre-malignant condition. By contrast, *cag*$^-$ *H. pylori* strains do not induce an intense host response, and do not promote IL-8 production. The exact equilibrium between *cag*$^+$ and *cag*$^-$ cells in a population will be determined by their relative fitness under each set of local conditions. Host genotype affects this equilibrium, as individuals possessing mutations that allow for high IL-1β (–31 C/C allele, –511 T/T allele) and TNF-α (–308 A/A allele) expression levels have a higher gastric pH, which downregulates *cagA* transcription, and provides selective pressure for *cag*$^-$ cells. Individuals colonized with *cag*$^+$ bacterial cells and who express high levels of IL-1β and TNF-α are more likely to develop atrophic gastritis and consequent hypochlorhydria, which increases risk of distal gastric cancer.

epithelial cells that are co-cultured with *H. pylori*[106] and within gastric mucosa of individuals infected with *H. pylori*[107,108]. COX-2 expression is further increased within pre-malignant (atrophic gastritis and intestinal metaplasia) and malignant (adenocarcinoma) lesions induced by *H. pylori*[109,110], and COX-inhibitors such as aspirin and other non-steroidal anti-inflammatory drugs have been shown to decrease the risk of distal gastric cancer[111,112]. *H. pylori* also activates phospholipase A_2, an enzyme that catalyses the formation of the prostaglandin precursor arachadonic acid, both *in vitro* and *in vivo*[113,114].

The inflammatory response induced by *H. pylori* leads to the release of mutagenic substances, such as metabolites of inducible nitric oxide synthase (iNOS), which promote oncogenesis[108,115]. Nitric oxide, generated by iNOS, can be converted to reactive nitrogen species that modify various cellular targets, including DNA and proteins.

Superoxide anion radicals generated by neutrophils also induce DNA damage through the formation of DNA adducts[99]. Serum levels of vitamin C, a scavenger of reactive oxygen species and nitrates, are inversely proportional to the prevalence of gastric cancer[116]. Eradication of *H. pylori* has been reported to raise gastric intraluminal ascorbic acid levels[117], so the presence of *H. pylori* also affects gastric mucosal antioxidant defence mechanisms.

Apoptosis. *H. pylori* has been associated with both increased and reduced levels of apoptosis in the gastric epithelium, depending on the human population studied (FIG. 3). *In vitro*, *H. pylori* reproducibly stimulates gastric epithelial-cell apoptosis[67,68,118,119]. *H. pylori* urease, an enzyme that generates ammonia and is present within the lamina propria of colonized individuals, has been shown to bind to class II MHC molecules on the surfaces of gastric epithelial cells *in vitro* and induce apoptosis[120]. *H. pylori* VacA has been reported to insert into mitochondrial membranes, induce cytochrome *c* release, and activate the caspase-3-dependent cell-death signalling cascade[69].

Another mechanism by which H. pylori can stimulate apoptosis is by inducing expression of the cell-surface receptor FAS and FAS ligand[67,118,121,122] (FIG. 3). Helicobacter infection of Ifn-γ-deficient and Fas-deficient mice is associated with reduced levels of inflammation and apoptosis compared with wild-type control mice[123–125]. This indicates that release of T_H1 cytokines induced by *H. pylori*, such as IFN-γ, might induce epithelial-cell apoptosis through a Fas-mediated pathway.

H. pylori has also been reported to induce apoptosis in gastric epithelial cells *in vitro* through activation of the transcription factor NF-κB[126] (FIG. 3). In colonic epithelial cells, however, NF-κB is negatively regulated by the nuclear hormone receptor peroxisome proliferator-activated receptor-γ (PPARγ)[127,128], and activation of PPARγ similarly inhibits *H. pylori*-induced activation of NF-κB and apoptosis in gastric cells[126] (FIG. 3). Putative endogenous PPARγ agonist ligands include the prostaglandin 15-deoxyΔ12,14-J_2 (15d-PGJ_2), a COX metabolite[129]. Another COX-2-generated metabolite, prostaglandin E_2 (PGE_2) also inhibits apoptosis[130]; in contrast to pro-inflammatory cytokines, prostaglandins might limit the apoptotic response that develops in response to *H. pylori*.

Different *H. pylori* strains have different effects on cellular turnover, and mucosal levels of apoptosis seem to vary substantially between individuals carrying *H. pylori*[67,131–133]. Two studies showed that gastric epithelial cells from people carrying *H. pylori cagA*$^+$ isolates have significantly higher proliferation rates, but lower apoptotic indices, than either *cagA*$^-$ or uninfected persons[134,135], although a recent study reported that apoptotic indices are increased within *H. pylori cagA*$^+$-colonized mucosa[136]. How could altered levels of apoptosis increase the risk of gastric cancer? Enhanced rates of apoptosis could potentially accelerate progression to atrophic gastritis, with a concomitant increase in the risk of distal gastric adenocarcinoma. By contrast, reduced rates of cell loss, especially when accompanied by hyperproliferation, could lead to a heightened retention of mutagenized cells, which might also predispose certain colonized individuals towards development of gastric cancer. On the basis of

the available data, it seems that apoptosis within mucosa colonized by *H. pylori* is regulated by host inflammatory mediators that modify the direct effect of the organism on epithelial cells, and the types and/or levels of mediator present (that is, T_H1 cytokines versus prostaglandins) might differentially alter cancer risk.

Host–bacterial equilibrium

H. pylori is able to send and receive signals from the gastric epithelium, allowing host and bacteria to become linked in a dynamic equilibrium[137]. The equilibrium is different for each colonized individual, based on both host and bacterial characteristics[40], which might explain why certain *H. pylori* strains augment the risk of carcinogenesis. For example, *cag*$^+$ strains induce an intense inflammatory response that involves production of IL-8. This leads to increased production of IL-1β and TNF-α, which inhibit acid production (FIG. 4) — especially in hosts with polymorphisms that promote high expression levels of these factors (TABLE 2). This combination of strain and host factors results in lower gastric acidity (higher gastric pH) than would occur in a person infected with a *cag*$^-$ strain or with polymorphisms that do not permit high expression levels of IL-1β or TNF-α. However, *cagA* transcription is reduced as pH rises[138,139], and the bacterial stimulus to the host diminishes, creating a negative-feedback loop that leads towards equilibrium. As *cag*$^+$ and *cag*$^-$ strains can coexist and recombine within the same host, the percentage of *cag*$^+$ strains is likely to vary over time, which will affect this equilibrium (FIG. 4).

Levels of mucosal proliferation are directly related to the intensity of inflammation, that is augmented by *cag*$^+$ strains. The ability of *H. pylori* in conjunction with inflammatory mediators to induce or attenuate apoptosis might also contribute to altered cellular turnover. An augmented inflammatory response induced by *cag*$^+$ strains in the gastric body, leading to decreased acid production, permits overgrowth of pH-sensitive bacteria, conversion of ingested *N*-nitrosamines to nitrites, and an increased risk of gastric cancer.

So, cancer risk is the summation of the polymorphic nature of the bacterial population in that host, the host genotype and environmental exposures (ingested nitrates), each affecting the level of the equilibrium. This deterministic model of the mucosal events related to carcinogenesis also has clinical and epidemiological ramifications. For example, people with polymorphisms associated with high levels of IL-1β expression and who are colonized by *cag*$^+$ strains might be most likely to derive benefit from *H. pylori* eradication, as such treatment could result in substantially reduced cancer risk.

GERD and oesophageal adenocarcinoma

The falling incidences of *H. pylori* carriage and gastric cancer in developed countries over the past century have been diametrically opposed by a rapidly increasing incidence of GERD and its sequelae. GERD is the strongest known risk factor for developing Barrett's oesophagus — a metaplasia of the distal oesophagus associated with an increased risk of oesophageal adenocarcinoma[140]. Among white males, the incidence of oesophageal adenocarcinoma has increased more than 350% since 1975 and its incidence is rising more rapidly than any other malignancy in the United States[141]. The relatively short (three-decade) timeframe over which the frequency of this cancer has increased indicates that an environmental factor might be involved. Could this factor be the falling incidence of *H. pylori*?

GERD is uncommon in geographical regions of the world in which most people are colonized by *H. pylori* (particularly *cag*$^+$ strains)[142]. GERD and its sequelae are increasing in incidence in Western countries[143], whereas the prevalence of *H. pylori* is falling[23]. In patients with duodenal ulcer disease (virtually always colonized with *cag*$^+$ strains), successful *H. pylori* eradication was associated with a doubling in the development of new-onset reflux oesophagitis over a 3-year period, compared with individuals who remained persistently colonized[144]. Carriage of *H. pylori* is associated with a significantly reduced risk of developing GERD, Barrett's oesophagus, and oesophageal adenocarcinoma, and the entire protective effect seems to be attributable to the presence of *cag*$^+$ strains[11,12,145–150].

How can the ability of *cag*$^+$ strains to enhance the risk of distal gastric cancer be reconciled with a presumed protective effect against GERD, Barrett's oesophagus and oesophageal adenocarcinoma? The location of inflammation within the gastric niche probably contributes to this dichotomy. By inhibiting parietal-cell function (FIG. 4) and/or inducing the development of atrophic gastritis, the severe inflammation in the acid-secreting gastric body (FIG. 2) induced by *cag*$^+$ strains (especially in patients with polymorphisms that cause increased expression levels of IL-1β and/or TNF-α) can blunt the high-level acid secretion necessary for the development of GERD and its sequelae. Recent clinical studies have shown that severe gastritis, atrophic gastritis and reduced acid production associated with *H. pylori* colonization significantly reduce the risk of GERD[11,151,152]. So, the interaction of *cag*$^+$ strains with their hosts has opposing effects on the risk of distal cancers (increases the risk of gastric adenocarcinoma) and proximal cancers (decreases the risk of oesophageal adenocarcinoma).

Conclusions

Analytical tools now exist — including genome sequences (*H. pylori* and human), measurable phenotypes (CagA seropositivity), and practical animal models — to discern the fundamental biological basis of *H. pylori*-associated neoplasia, which should have direct clinical applications. For example, elucidating the role of specific proteins (CagA, VacA) secreted by *H. pylori* in the pathogenesis of gastric adenocarcinoma might aid in vaccine development in high-risk populations. It is important to gain more insight into the pathogenesis of gastric adenocarcinoma induced by *H. pylori*, not only to develop more effective treatments for this common cancer, but also because it might serve as a model for the role of chronic inflammation in the genesis of other malignancies, such as ulcerative colitis-associated colon cancer.

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Online links

DATABASES

The following terms in this article are linked online to:
CancerNet: http://cancernet.nci.nih.gov/
gastric adenocarcinoma | non-Hodgkin's lymphoma | oesophageal adenocarcinoma
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
caspase-3 | CCK-β | *CDH1* | COX-1 | COX-2 | cytochrome *c* | E-cadherin | gastrin | gastrin receptor | IFN-γ | IL-1 | IL-1β | IL-8 | IL-10 | iNOS | NF-κB | p27 | phospholipase A2 | PPARγ | RAS | TNF-α | *TP53*
Medscape DrugInfo:
http://promini.medscape.com/drugdb/search.asp
aspirin

FURTHER INFORMATION

AstraZeneca's Gastroesophageal Reflux Disease (GERD) information resource centre: http://www.gerd.com/
GERD online: http://gerdonline.itgo.com/index.html
Medline Plus web site on oespophageal cancer:
http://www.nlm.nih.gov/medlineplus/esophagealcancer.html
OncoLink outline of stomach cancer:
http://www.oncolink.com/templates/types/article.cfm?c=5&s=14&ss=105&id=1736
Oncology Forum's web site on oesophageal cancer:
http://www.oncologychannel.com/esophagealcancer/
The Canadian *H. pylori* web site:
http://www.canadianhp.com/english/gastric_cancer.html
The US Centers for Disease Contol's factsheet on *H. pylori* and peptic ulcer disease: http://www.cdc.gov/ulcer/md.htm
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