



Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications

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Humans have been colonized by *Helicobacter pylori* for at least 50,000 years and probably throughout their evolution. *H. pylori* has adapted to humans, colonizing children and persisting throughout life. Most strains possess factors that subtly modulate the host environment, increasing the risk of peptic ulceration, gastric adenocarcinoma, and possibly other diseases. *H. pylori* genes encoding these and other factors rapidly evolve through mutation and recombination, changing the bacteria-host interaction. Although immune and physiologic responses to *H. pylori* also contribute to pathogenesis, humans have evolved in concert with the bacterium, and its recent absence throughout the life of many individuals has led to new human physiological changes. These may have contributed to recent increases in esophageal adenocarcinoma and, more speculatively, other modern diseases.

Helicobacter pylori, Gram-negative bacilli that colonize the human stomach, are the main cause of peptic ulceration, gastric lymphoma, and gastric adenocarcinoma, the second leading cause of death from cancer worldwide. They also may contribute to other conditions, including iron and vitamin B12 deficiencies, idiopathic thrombocytopenic purpura (ITP), and growth retardation in children. *H. pylori* colonization occurs in childhood and persists throughout life, causing disease mainly in adults (1, 2). However, despite the fact that about half the world's population carries *H. pylori*, only a small proportion develop ulcers or gastric cancer. This raises a number of questions, including how has *H. pylori* adapted to persistently colonize humans? and why (and how) does it cause disease in only a minority of those colonized?

The other side of this coin is that although humans have been colonized for millennia by *H. pylori*, it is now disappearing (2, 3). During the time period over which *H. pylori* has gradually disappeared from some populations, including much of the USA and western Europe, other diseases have become more prevalent. For example, there is an increased incidence of gastroesophageal reflux disease (GERD) and its complications; obesity and its associated diseases, including type 2 diabetes; and atopic and allergic diseases, including asthma. These observations also raise a number of questions, including how have we adapted to *H. pylori* colonization over millennia? and does the absence of *H. pylori* cause any physiologic or immunologic imbalances that contribute to diseases of modern life? As we discuss in this Review, recent extensive genomic and molecular analyses have shed some light on these issues.

***H. pylori* and humans: an ancient relationship**

Helicobacters inhabit the gastrointestinal tract of many mammals and birds, which often possess their own unique gastric *Helicobacter* species. These species are mostly host specific (ref. S1; available online with this article; doi:10.1172/JCI38605DS1), implying coevolution of the bacteria with their hosts. By comparing nucleotide sequences of different strains and measuring maximal in vivo mutation rates, it is possible to calculate the minimal time that *H. pylori* and its host have shared a common ancestor (4). Genetic

diversity among *H. pylori* strains decreases with distance from East Africa, just like genetic diversity decreases among humans (Figure 1) (5). Taken together these data show that *H. pylori* has coevolved with humans, at least since their joint exodus from Africa 60,000 years ago and likely throughout their evolution.

***H. pylori* adaptations to human colonization**

H. pylori colonizes our stomachs in childhood and persists throughout our lives (2). This implies near perfect adaptation to the niche and an ability to evade the human immune response (S2). Its spiral shape and flagella allow it to corkscrew through the gastric mucus gel, and numerous adhesins enable selective adherence to the epithelium. *H. pylori* has multiple mechanisms for protection against gastric acid (6); notably, 15% of its protein content comprises preformed cytoplasmic urease. When the external pH is less than 6.5, a specific channel opens in the bacterial cytoplasmic membrane, allowing ingress of urea (7). The ammonia produced by urea hydrolysis neutralizes the periplasm, allowing maintenance of the cytoplasmic membrane potential (7).

Like many human commensal bacteria, *H. pylori* has evolved specific mechanisms to avoid stimulating the immune response. For example, innate immune recognition by several TLRs is attenuated for *H. pylori* (8, 9) (S3). Despite this, colonization is associated with inflammatory cell infiltration into the gastric mucosa (termed gastritis). Different *H. pylori* strains induce varying degrees of gastritis, reflecting their individual abilities to interact with the host; some possess "host interaction" (also known as virulence) factors (1). How these factors result in disease is becoming better understood (S4), although the benefit to *H. pylori* of possessing them remains less clear. One possibility is that the epithelial changes *H. pylori* causes (directly and perhaps also through inflammation) allow increased nutrient delivery to the bacterium. Alternatively, or additionally, these host interactions may induce niche modification, resulting in better conditions for survival of *H. pylori* or worse conditions for survival of competing bacteria.

***H. pylori* host interaction factors**

CagA and the *cag* pathogenicity island. We have previously written about *cag* pathogenicity island-associated (PaI-associated) effects (1), but research in this field has advanced, particularly that con-

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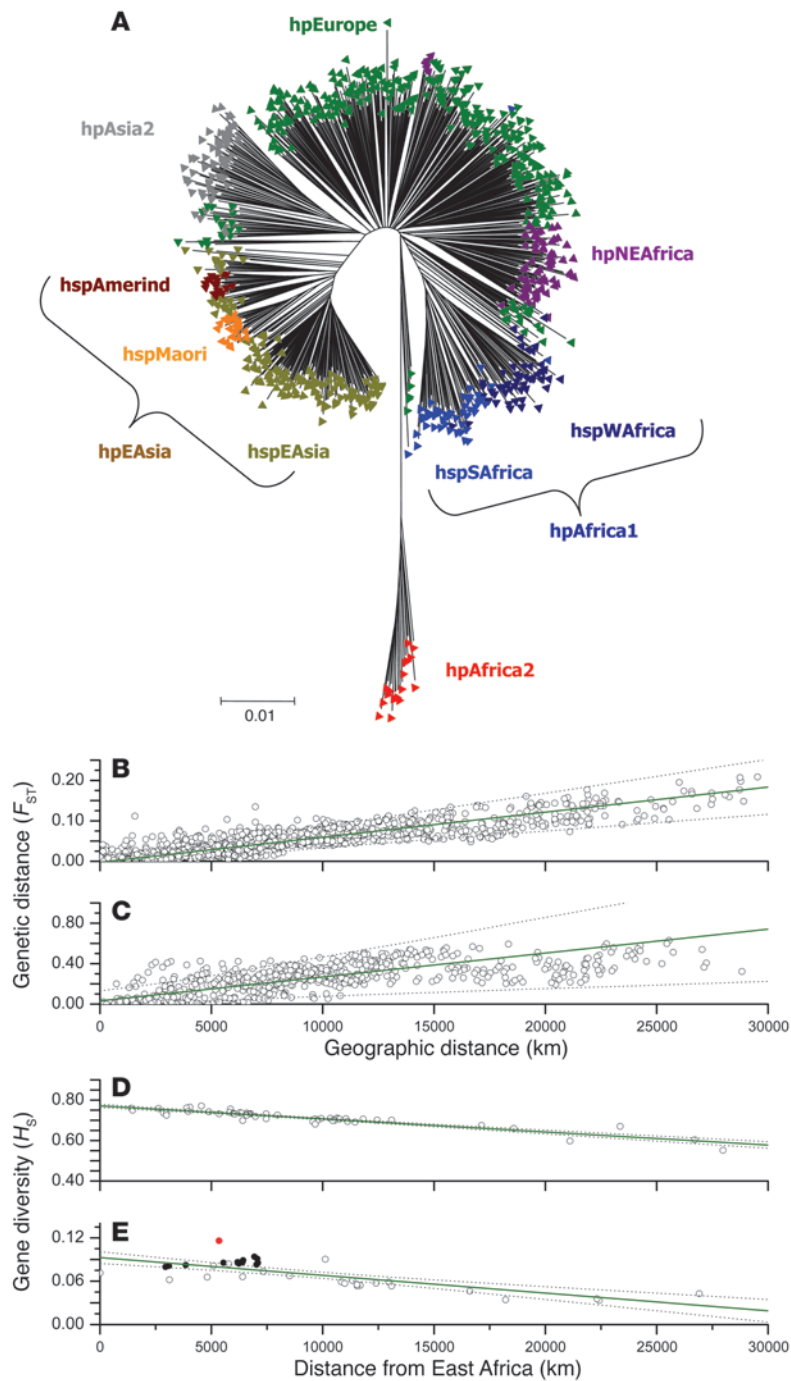


Figure 1

Phylogeography of *H. pylori*. (A) Genetic diversity. Neighbor-joining tree (Kimura 2-parameter) of 769 concatenated sequences from *H. pylori*, color-coded according to population assignment by structure into the populations *H. pylori* Europe (hpEurope), *H. pylori* Asia 2 (hpAsia2), *H. pylori* North East Africa (hpNEAfrica), *H. pylori* Africa 2 (hpAfrica2), *H. pylori* Africa 1 (hpAfrica1), with subpopulations *H. pylori* West Africa (hspWAfrica) and *H. pylori* South Africa (hspSAfrica), and *H. pylori* East Asia (hpEAsia), with subpopulations *H. pylori* American Indian (hspAmerind), *H. pylori* Maori (hspMaori), and *H. pylori* East Asia (hspEAsia). (B–E) Parallel geographic patterns of genetic diversity in humans and *H. pylori*. (B and C) Genetic distance in humans (B) and *H. pylori* (C) between pairs of geographic populations versus geographic distance between the two populations. F_{ST} is the proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance. (D and E) Average gene diversity (H_s) in humans (D) and *H. pylori* (E) within geographic populations versus geographic distance from East Africa. $r^2 = 0.77$ (B); $r^2 = 0.47$ (C); $r^2 = 0.85$ (D); and $r^2 = 0.59$ (E). Confidence intervals are indicated by dotted lines. Reproduced with permission from *Nature* (5).

cerning how effects are localized within the epithelial cell (Figure 2) (10–18) (S5), how the CagA effector protein varies between strains (Table 1) (19–23), and how CagA can directly induce carcinogenesis. In *H. pylori* strains, the *cag* PaI may be present, absent, or disrupted and thus nonfunctional. It is most commonly present and functional (24), containing approximately 30 genes, including those that collectively encode a type IV secretion system (T4SS) — a hollow conduit that connects the cytoplasm of the bacterial and host epithelial cells (10) (S6). This is beautifully designed for the human stomach: an antigenically variable, acid-stable structural protein (CagY) coats the “syringe,” conferring stability and allow-

ing evasion from the host immune response (25, 26). Subsequent contact of the tip protein (CagL) with the epithelial cell and delivery and activation of the effector protein (CagA) stimulate local signaling and effects at the site of attachment — focal adhesions between epithelial cells (11) (Figure 2). CagA may also have more widespread cellular effects, including activation of NF- κ B (15) (Figure 2). NF- κ B activation also is stimulated by the presence of the T4SS itself, through recognition of small quantities of bacterial peptidoglycan products by the intracellular host epithelial pattern-recognition receptor nucleotide-binding oligomerization domain-containing 1 (Nod1) (14).



Table 1
Polymorphisms in *H. pylori* CagA affect the magnitude of its effects

Geographic locale	CagA type ^A				Bacterial effects		Association with gastric cancer
	Type A TPM	Type B TPM	Type C TPM	Type D TPM	Epithelial cytoskeletal changes	Epithelial IL-8 secretion	
Western	Absent ^B	Absent	Absent	Absent	–	+	±
Western/East Asian	Disrupted ^C	Disrupted	Disrupted	Disrupted	–	+	±
Western	1	1	1	1	++	++	++
Western	1	1	2	Absent	+++	+++	+++
Western	1	1	3	Absent	++++	++++	++++
East Asian	1	1	Absent	1	+++++	+++++	+++++

^AIn most *H. pylori* strains, CagA has single type A and B TPMs, although occasionally these are multiple. Additionally, Western CagA (in European and American strains) commonly has from 1–3 active type C TPMs (19, 20). The more type C TPMs, the more heavily phosphorylated CagA is within the cell, leading to more profound effects and a stronger association with gastric cancer (19–23). East Asian (Japanese and Chinese) strains have a single type D motif instead of the C motifs. This is associated with stronger SHP-2 binding and activation and more profound cellular effects (19, 22). ^BSome Western *H. pylori* strains have no CagA. ^CSome Western and East Asian *H. pylori* strains have either disrupted CagA forms or a disrupted *cag*-encoded T4SS. –, no effect; ±, uncertain association; +, ++, +++, +++++, and ++++++, increasing effects or strength of association.

CagA is polymorphic (Table 1); in particular, CagA from different *H. pylori* strains has different numbers and types of activating tyrosine phosphorylation motifs (TPMs), leading to different effects on cellular signaling and differing risks of disease (19–23). The D-type TPM found in East Asian strains binds to Src homology 2 domain-containing tyrosine phosphatase 2 (SHP-2) strongly and stimulates very marked cellular effects (19, 22, 26). This may, in part, explain the high rate of *H. pylori*-associated disease in Japan and parts of China.

CagA appears to be centrally important in *H. pylori*-induced gastric carcinogenesis, regardless of inflammation status. Among transgenic mice engineered to express CagA constitutively, some developed B cell lymphomas and some developed gastric adenocarcinomas (27). That there was no gastritis implies that the underlying mechanism did not involve chronic inflammatory damage, although early time points were not examined. The implications for human gastric intestinal-type carcinogenesis, which appears to arise on a background of inflammation through a stepwise progression of gastritis-atrophy-metaplasia-carcinoma (S7), remain to be fully determined. Eradication of *H. pylori* in the human atrophic stomach does not greatly reduce the proportion of people who develop cancer over a 5-year time frame (28), implying that CagA effects must be mediated relatively early in the carcinogenic process.

The vacuolating protein, VacA. Virtually all strains of *H. pylori* possess the gene *vacA*, and nearly all produce a VacA protein (29). However, only about 40% make the most active form. VacA, an exotoxin comprising two subunits, p33 and p55 (30) (S8), is specifically adapted to the stomach, being activated by acid and then becoming resistant to acid and pepsin degradation (31). It forms pores in epithelial cell membranes, explaining many of its effects, including vacuolation (32) (S9). VacA binds to receptor-like protein-tyrosine phosphatase- α (RPTP α) and RPTP β (33) (S10); transgenic mouse studies show that these receptors are required for VacA-induced gastric ulceration (33).

Recently, very specific VacA effects have been demonstrated in vitro on T cells. VacA associates specifically with β_2 integrin on T cells (34), blocks calcium influx, and thereby inhibits activation of the transcription factor nuclear factor of T cells by the calcium-dependent phosphatase calcineurin (35) (S11). This inhibits T cell

proliferation and induces cell-cycle arrest (S12). The specificity of these effects implies physiological importance, but thus far none has been demonstrated in vivo, either in rodents (perhaps expectedly as the different integrin profile on their T cells precludes VacA activation) or humans.

vacA varies among *H. pylori* strains in three major regions: a 5' region, encoding the signal peptide and mature protein N-terminus (s1 or s2); an intermediate region, encoding part of the p33 subunit (i1 or i2); and a mid region, encoding part of the p55 epithelial cell-binding subunit (m1 or m2) (36, 37). The s1/i1/m1 form of VacA is fully active, and the s2/i2/m2 form is inactive, but intermediate forms exist and are more common in many human populations (38) (S13). The s1/i1/m2 form is active on fewer epithelial cell lines than the s1/i1/m1 form, since m2 forms bind to a narrower range of epithelial cells (39). The s1/i2/m2 form is nonvacuolating (37), although the mechanism whereby the i2 region confers nontoxicity is unknown. Other forms of the toxin are rare. Strains with *vacA* encoding active s1/i1/m1 and s1/i1/m2 forms are strongly associated with gastric adenocarcinoma and associated (albeit more weakly) with peptic ulceration (23, 37) (S13–S16). These forms are almost universal in Japan and northern China, perhaps contributing to the high rate of gastric cancer in these regions (S17).

How active forms of VacA predispose to disease remains unclear. In mouse models, VacA aids colonization, and purified VacA causes peptic ulceration (40). In the *H. pylori*-infected Mongolian gerbil, toxigenic strains increase ulcer and cancer risk but VacA is not a prerequisite (41). These effects appear independent of inflammation in rodents. In humans, active VacA types have been strongly associated with precancerous gastric atrophy and hypochlorhydria, even when controlling for *cag* status (42). Furthermore, in patients with hypochlorhydria, the toxigenic *H. pylori* strains have been shown to have been acquired in childhood, implying that they predate and so may cause the atrophy (43). In vitro, VacA inhibits parietal cell function (44), and loss of parietal cells is the hallmark of gastric atrophy. Whether VacA inhibits acid production directly in vivo and, if so, how this may lead to parietal cell loss, remains unknown.

Adhesins. Adhesion is a prerequisite for both *H. pylori* colonization and disease induction, and some strains adhere better than others to epithelial cells (45) (S18). The repertoire of functional adhesins

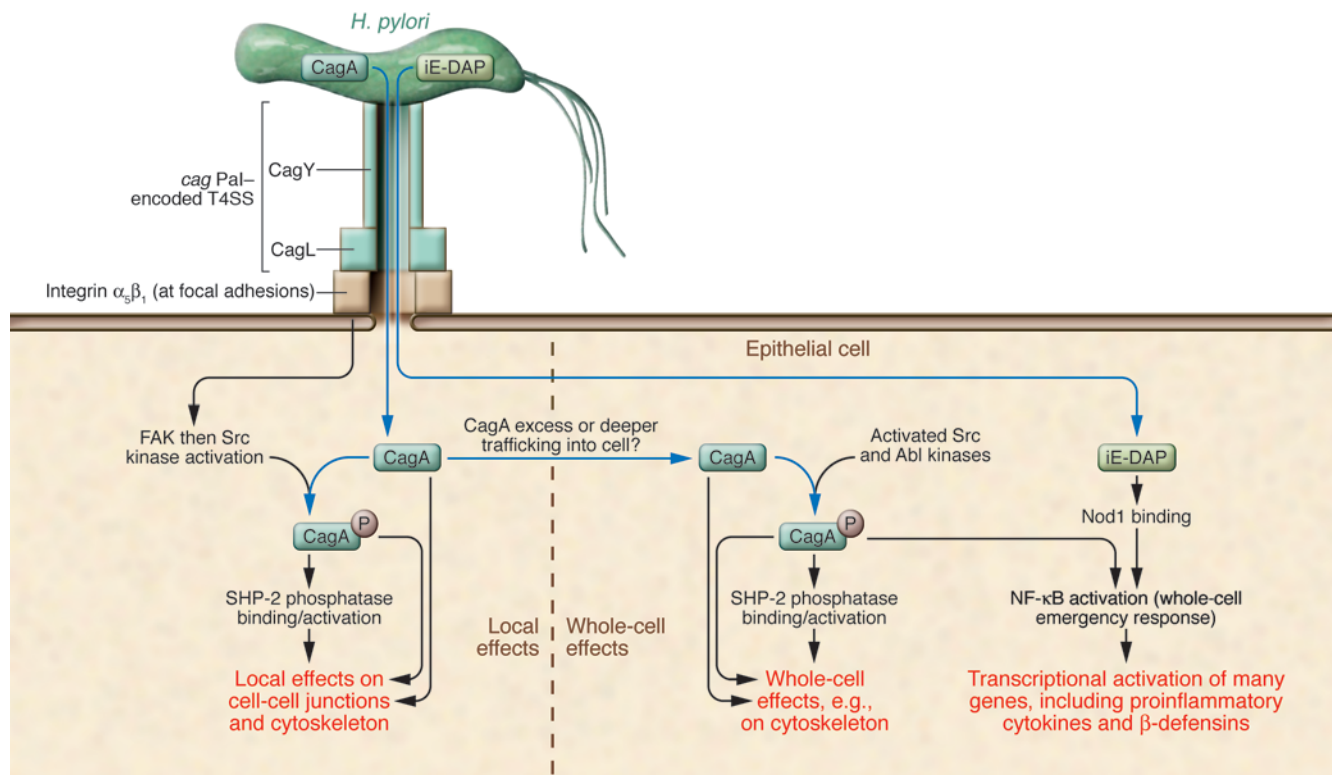


Figure 2

CagA phenotypes and variation. Local and whole-cell effects of the *H. pylori* *cag*-encoded T4SS and its major effector protein, CagA. *H. pylori*, with an intact *cag* Pal, forms a T4SS, which injects CagA into epithelial cells (10). The T4SS tip protein, CagL, binds to and activates integrin $\alpha_5\beta_1$, resulting in local activation of focal adhesion kinase (FAK) and then Src kinase (11). Activated kinases phosphorylate CagA at specific tyrosine residues, in turn activating local Src homology 2 domain-containing tyrosine phosphatase 2 (SHP-2) and therefore local signaling (12, 13, 125). A soluble component of bacterial peptidoglycan, γ -D-glutamyl-*meso*-diaminopimelic acid (iE-DAP) also enters the cell and is recognized by the intracellular innate immune pattern-recognition receptor Nod1, leading to stimulation of NF- κ B (14). Furthermore, phosphorylated CagA itself, possibly when in excess or when trafficked deeper into the cell, also may activate NF- κ B and have other whole-cell effects (8, 15–17). Blue arrows indicate *H. pylori* components, black arrows indicate epithelial cell components, and red text indicates cellular effects.

appears to have been driven by human evolution (46). The cellular adhesin most closely and consistently associated with disease is blood group antigen-binding adhesin A (BabA; encoded by *babA2*). This recognizes Lewis Blood Group epitopes on epithelial cells and is often found in *H. pylori* strains associated with gastric cancer (45) (S18). In animal models, BabA-producing strains induce more inflammation and in vitro they cause epithelial cells to release more IL-8 than strains that don't produce BabA (S18). Close adherence allows better delivery of other interaction factors (47) (S19).

Duodenal ulcer-promoting gene A. Duodenal ulcer-promoting gene A (*dupA*) is so named because some studies suggested a positive association with duodenal ulceration and a negative association with gastric cancer (48, S20–S22). However, others have shown associations with both conditions (S20), as might be expected for a factor associated with increased IL-8 release and gastritis in vivo (48). Since *dupA* has homology to *virB4*, it may be the ATPase of an as yet uncharacterized T4SS (48).

***H. pylori* diversity and genetic adaptation to its niche**

One of the most remarkable features of *H. pylori* is its enormous genetic diversity (4, 5, 49). Although most genomes range from 1.5 to 1.8 Mb (50, 51) (S23–S25), diversity among strains includes variation in the complement of genes, chromosomal gene order,

deployment of repetitive DNA, sequence variation (on average 6%) in conserved genes, homoplasies, status of phase-variable genes, complement of restriction-modification loci, and mobile DNA (52). Despite this diversity, similarities between strains based on human-population origins are maintained (53). *H. pylori* generates diversity through point mutation; intragenomic and intergenomic recombination, phenomena that are enhanced by the presence of ROS and reactive nitrogen species (RNS) from the inflammatory lesions induced by *H. pylori*; and second-order selection, involving variation in mutator genes (49, 54, 55) (S26–S29) (Figure 3). In all, this creates a nonlinear system for diversification (S30).

That *H. pylori* interactive factors are linked with disease implies that they are a fixed characteristic, but this is not the case (Figure 4A) (56). Variation in relevant genotypes through intragenomic recombination (e.g., number of CagA TPMs) (43, 57) (Figure 4B) likely reflects local selection for particular *H. pylori* phenotypes (Figure 2). *H. pylori* strains can also adapt (or repair) by recombination with other strains or with clonal variants of the same strain (58). Examples of presumed adaptation include strains exchanging *cagA* alleles or losing all or part of the *cag* Pal during infection of an individual human (43) (Figure 4B). Intergenomic recombination with other strains may also affect other host interaction factors, e.g., leading to changes in VacA genotype and phenotype (43). One



possibility is that multiple minor variants of a strain may survive in an individual stomach, each acting as potential reservoirs of genetic elements for their cohabitants, with the fittest dominant clone(s) being selected by environmental pressures (1) (43, 58) (S31). Implications for pathogenesis are unclear but likely important.

The growing complexity of *H. pylori* variation has been exposed by published whole genomic sequences of six isolates to date (50, 51) (S23–S25). However, if the phase variations and similar polymorphisms are truly independent, then the number of potential variants is enormous, resembling the “quasispecies” populations of RNA viruses, such as HIV and HCV, two other organisms that persist in their hosts (1) (S31). As genome sequencing costs continue to fall, parallel sequencing of isolates from different parts of the stomach from individual hosts would enhance understanding of functional diversity in relation to host phenotypes; multiple (competing) strains often are present within a host but cooperate through quorum sensing and recombination, among other properties (43, 49) (S32, S33). A related approach is to use high-throughput sequencing to understand the abundance and diversity of other bacterial species (as well as their genes) in persons with and without *H. pylori* in both health and specific disease states.

The human immune response to *H. pylori*

An immune response to a specific microbe is multifactorial: although largely determined by host genetics, it is heavily influenced by environmental factors (including other microbes) and may be influenced by the target microbe itself. Although *H. pylori* has evolved to minimize stimulation of innate immunity, such activation does occur and is important in pathogenesis (14, 59, 60) (S34). *cag* PaI-induced epithelial cell IL-8 secretion is an important initiator of the immune response, and ongoing IL-8 secretion and consequent neutrophil infiltration are important in the pathogenesis of peptic ulceration and probably gastric carcinoma (61, 62).

The importance of host innate immune factors in *H. pylori*-associated disease is underlined by studies of their genetic polymorphism. For example, functional polymorphisms in the genes encoding IL-1 β and IL-1 receptor antagonist result in host-specific differences in IL-1 β secretion in response to microbes and to differences in gastric cancer risk (63, 64) (S35). Polymorphisms in other genes leading to increased proinflammatory or reduced antiinflammatory cytokine release, as well as genes encoding some TLRs, also increase risk of *H. pylori*-associated disease (S35, S36).

Besides stimulating innate immunity, *H. pylori* stimulate strong humoral and cell-mediated acquired immune responses (S36). However, humoral responses are not involved in protection, even in mouse models of infection (65). In contrast, cell-mediated responses play a role in both clearance of the organism and pathogenesis in animal models (66) (S37) and are likely to play a role in pathogenesis in humans. The predominant human T cell response to *H. pylori* is Th1 mediated and is therefore associated with proinflammatory cytokine release and profound macrophage activation (67) (S38) (Figure 5). Experimentally infected mice, with strong Th1 responses, develop intense gastritis but have low gastric bacterial loads, whereas the reverse is observed in mice that mount a predominantly Th2 response (68). Manipulation of mouse immune responses toward Th1 increases inflammation and gastric atrophy; manipulation toward Th2 reduces them (69) (S39). The direct importance of the acquired immune response in pathogenesis is evidenced by the observation that the Th1 cytokine IFN- γ induces gastric atrophy in mice, even in the absence of *Helicobacter*

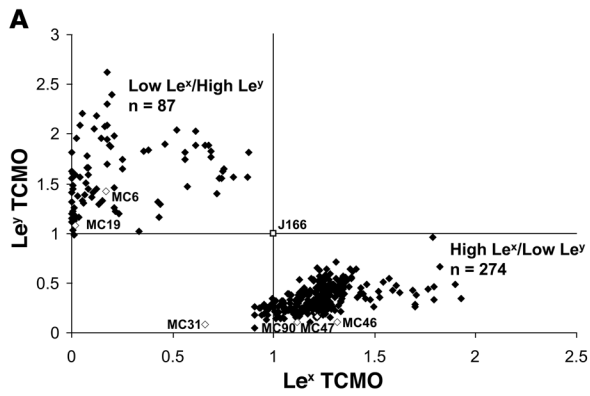
species, although infection potentiates this effect (S40). In humans, Th responses are less polarized than in mice, and patients with peptic ulcers have both stronger Th1 and Th2 gastric responses to *H. pylori* antigens than do colonized patients without ulcers (70). At a population level, the IgG subclass response to *H. pylori* suggests a Th2 bias in parts of Africa and a Th1 bias in Japan and the United Kingdom, possibly contributing to the lower disease prevalence in Africa (71) (S38, S41).

To persist, *H. pylori* must evade the immune response, and persistence is essential for pathogenesis, since ulcers and cancer arise decades after acquisition of the organism. Most *H. pylori* live superficial to the epithelial cell layer, and immune effectors may not easily access and function in the gastric mucus niche. *H. pylori* itself produces factors, including CagA and VacA, that decrease T cell activation in vitro (35) (S42). However, the main physiologic mechanism in humans for controlling Th responses is through Tregs. Tregs have been found in the *H. pylori*-positive gastric mucosa, along with elevated levels of the immunosuppressive cytokines IL-10 and TGF- β (72) (S41, S43). Furthermore, Tregs suppress *H. pylori*-induced epithelial cell production of IL-8- and *H. pylori*-specific memory cell responses (73) (S44). *IL10*^{-/-} mice develop intense local gastritis following infection with *Helicobacter* species and then clear the infection, showing the importance of this cytokine in both controlling inflammation and promoting persistence (S45). Collectively, these data suggest that *H. pylori*-responsive gastric Tregs are important both in promoting persistence and in controlling inflammation (and disease) (S36) (Figure 5). Importantly, patients with peptic ulcer disease have many fewer gastric mucosal Tregs than *H. pylori*-positive patients without peptic ulcers (70).

H. pylori-human interactions in the pathogenesis of specific diseases

Pathogenesis of peptic ulceration: a modern disease. Virulent strains of *H. pylori* predispose to peptic ulceration, and treatment to eradicate *H. pylori* heals ulcers and largely prevents recurrence. However, pathogenesis of duodenal ulcers and gastric ulcers differ (S4); duodenal ulcers arise on a background of *H. pylori*-induced antral-predominant gastritis; this causes hypergastrinemia and high levels of acid production from the healthy gastric corpus following meal or hormonal stimulation (mechanisms described in our previous review (1). The duodenum develops gastric metaplasia, presumably in response to high acid load, and unlike the normal duodenal mucosa, can be colonized by *H. pylori*, with consequent inflammation and ulceration (74). In contrast, gastric ulcers are associated with *H. pylori*-induced pan- or corpus-predominant gastritis, with normal or reduced acid levels, and ulcers usually arise at the junction of the antral and corpus mucosa, an area of intense inflammation (S47). *H. pylori*-induced duodenal ulcers commonly occur in middle age, whereas gastric ulcers typically affect older people.

Both gastric and duodenal ulcers are “modern” diseases (Figure 6A). Gastric ulcer disease arose in the 19th century in the USA and Europe, and its prevalence peaked in the early twentieth century; duodenal ulcer disease arose and peaked 10–30 years later (75) (S46). The fall in prevalence in these diseases likely is due to the falling prevalence of *H. pylori*: as living standards changed, cohorts of children became progressively less likely to acquire the organism (2). The reasons underlying the emergence of peptic ulcer disease are uncertain, yet worthy of analysis. One possible contributor is cigarette smoking, although this became popular too late to fully explain the phenomenon, and other forms of tobacco smok-



B

<i>mutY</i> codon number	Sequence				Number of strains	Presumed ORF
	91	92	93	94		
TTA	AAA	AAA	AAA	AGT	398	In
TTA	AAA	AAA	AAA	_GTG	1	Out
TTA	AAA	AAG	AAG	AGT	12	In
TTA	AAA	AGA	AGA	AGT	2	In
Amino acid	L	K	K/R	S/V		

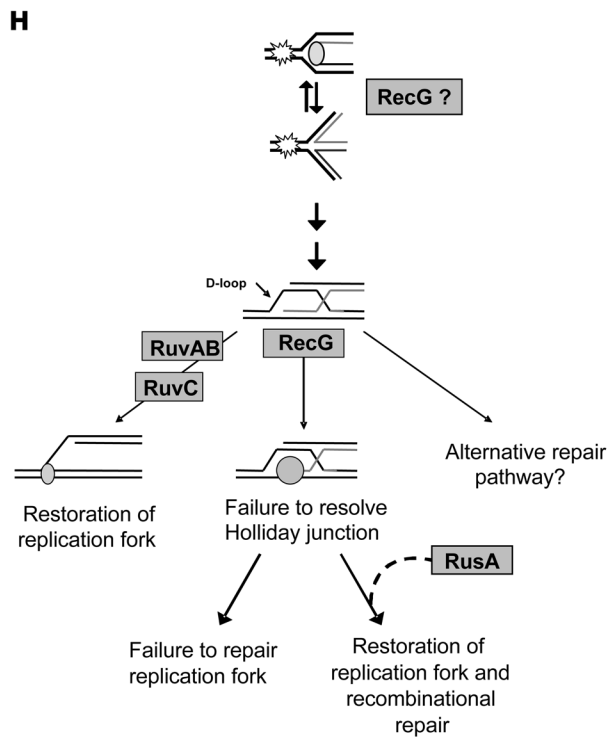
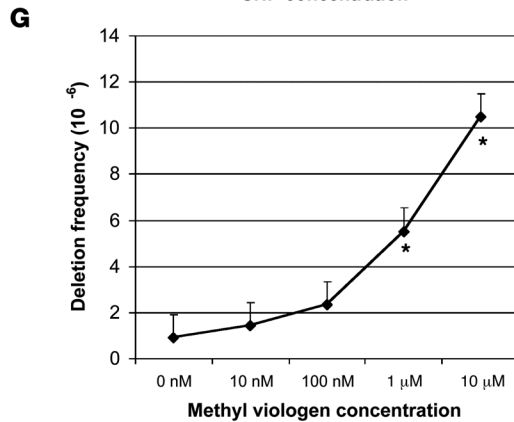
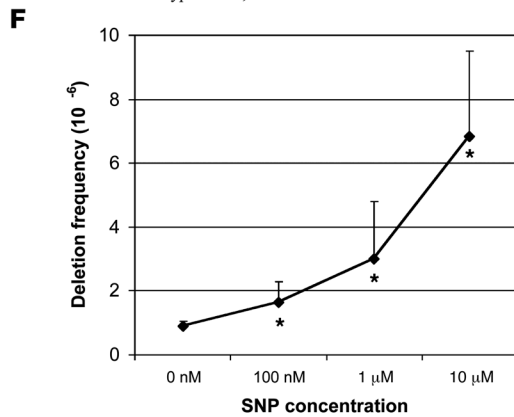
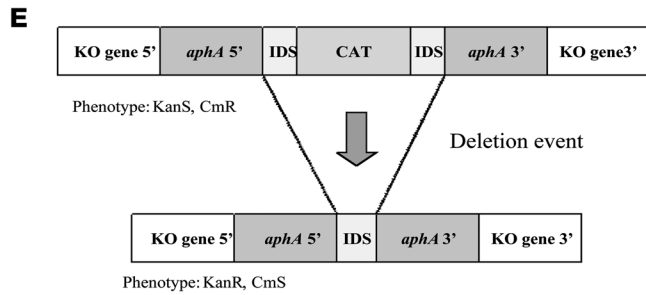
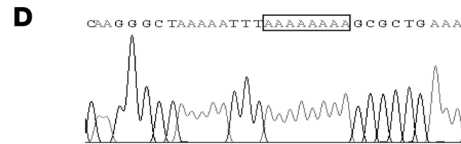
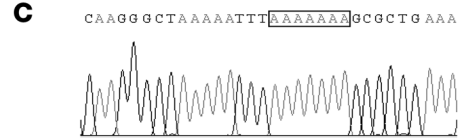




Figure 3

Mechanisms for generating genetic diversity in *H. pylori*. (A) Slipped-strand mispairing in a homopolymeric tract, leading to phase variation in surface antigen expression. Lewis (Le^x and Le^y) expression in single colonies of an *H. pylori* strain were determined by ELISA, with transformed converted mean OD units (TCMO) calculated. Two major population groupings were observed, based on frame-status of *futC*, the gene encoding α -1,2-fucosyltransferase. Reproduced with permission from *Microbes and infection* (S28). (B–D) Polymorphisms in the gene *mutY*, which encodes DNA glycosylase MutY. (B). Genotypes of 413 *mutY* homonucleotide tracts from the *H. pylori* multilocus sequence typing (MLST) database. (C) Chromatograph of the *mutY* homonucleotide with seven adenines (out of frame). (D) Chromatograph of in-frame *mutY* (eight adenines). When *mutY* is out of frame, the *H. pylori* cells have a mutator phenotype, augmenting genomic point mutation. Reproduced with permission from *Journal of bacteriology* (S29). (E–G) Effect of exposure to RNS and ROS in frequency of deletions involving DNA repeats. (E) Deletion cassette. Cells with this were exposed to RNS (F) or ROS (G) and deletion frequency was calculated. Asterisks indicate significant ($P < 0.05$) increases in point mutation frequencies when compared with those calculated for cells incubated in 0 nM of SNP or methyl viologen. *aphA*, gene encoding aminoglycoside resistance; CAT, chloramphenicol acetyl transferase; CmR, resistant to chloramphenicol; IDS, identical repeat sequence; KanS, susceptible to kanamycin. Reproduced with permission from *FASEB journal* (S27). (H) Proposed mechanism of RuvB/RecG Holliday junction resolution. Two *H. pylori* DNA processing pathways may compete for the same Holliday junction intermediate, which can branch migrate (left pathway) because of RuvAB and be resolved by RuvC, restoring replication fork, enabling loading of the replisome (small oval). Alternatively, (middle pathway) RecG (gray circle) can branch migrate Holliday junctions, but without resolution, leading to cell death. Addition of Rusa restores the RecG pathway, leading to recombinational repair. The incomplete *recG* pathway reflects a tension between DNA repair and antirepair. Reproduced with permission from *Journal of bacteriology* (S26).

ing were widespread before ulcers became common. A second possibility is increased *H. pylori* virulence. *H. pylori* can rapidly adapt and evolve in the stomach if multiple strains are present, which may lead to downregulation of interaction with the host (43, 58). If gradual decreases in transmission led to colonization, with single strains becoming more common, the facility of *H. pylori* to downregulate interaction (virulence) may have diminished. A variation of this hypothesis is that acquisition of two different strains may allow colonization of both antrum and corpus, with little physiological disturbance, whereas infection with a single antral-colonizing strain could lead to duodenal ulceration. A further variation is that diminished transmission may have increased the likelihood of acquiring strains from strangers, not genetically related family members, with greater mismatch. Finally, changes in immunity may be important. Patients with peptic ulceration have an impaired gastric mucosal Treg response, compared with *H. pylori*-positive persons without ulcers (70). Gradual reduction in colonization with other microbes over the late 19th and early 20th century or improvement in nutrition may have led to a less mature Treg response in some hosts, predisposing to increased gastric inflammation and peptic ulceration (Figure 5).

Pathogenesis of gastric adenocarcinoma: still enigmatic. Gastric adenocarcinoma has been classified into two types: the more common intestinal and the less common diffuse type, which is more rapidly aggressive and tends to occur in younger adults (S48). Diffuse cancer sometimes arises on a background of simple *H. pylori*-induced gastritis, but this has not been extensively studied.

Intestinal-type gastric cancer arises in the context of pan- or corpus-predominant gastritis and the resultant hypochlorhydria, explaining why patients with duodenal ulcers (which are associated with an antral-predominant gastritis and high-stimulated acid production) are partially protected against gastric adenocarcinoma (76). Intestinal-type gastric cancer may be the end stage in a progression from simple gastritis to gastric atrophy, metaplasia, dysplasia, and carcinoma (S7). The crucial step is atrophy (28). Although *H. pylori* treatment in the atrophic stomach may prevent some cancer cases (perhaps by preventing further development of atrophy or by partially resolving inflammation that is directly DNA damaging), the more important fact is that cancer can, and frequently does, still develop (58) (S50). In contrast, removal of *H. pylori* before the development of atrophy appears to prevent carcinogenesis (28). Gastric atrophy due to autoimmune gastritis also is a risk factor for gastric adenocarcinoma, further implying that atrophy is important, rather than *H. pylori* per se (S49). Thus, the main role of *H. pylori* in intestinal-type gastric cancer appears to be the induction of atrophy, implying that research should concentrate on this process.

How atrophy leads to cancer remains unclear. One hypothesis reflects that hypochlorhydria allows the overgrowth of bacteria other than *H. pylori* that release DNA-damaging ROS and RNS (77). A related theory suggests that the free radicals from inflammatory cells (particularly neutrophils) may be the carcinogenic agents. The compensatory hypergastrinemia accompanying atrophy may also contribute, since gastrin promotes proliferation; transgenic hypergastrinemic mice develop accelerated cancer in response to other agents (78). A further theory, based on several lines of evidence (79, 80), suggests that stem cells may be a direct target of *H. pylori* in the atrophic stomach, even though *H. pylori* are less numerous there than in the healthy stomach. To understand the origin of cancer cells in the *Helicobacter*-infected mouse model, marker studies were conducted in irradiated mice, who subsequently underwent bone marrow transplantation (81). This work showed that many gastric adenocarcinoma cells (in several cell lineages) were derived from bone marrow cells, suggesting that these may replace gastric stem cells lost in the development of atrophy. The bone marrow stem cells may be genetically more unstable, or may simply occupy a less well-protected niche, predisposing to mutation. Whether some gastric cancer in humans arises from bone marrow stem cells is unknown.

The theory that cancer in humans develops from the atrophy caused by *H. pylori* is challenged by *cagA*-transgenic mice that develop adenocarcinoma-like histopathological changes, without inflammation or atrophy (27). However, in humans CagA must induce a chain of events rather than being the final step in cancer causation, otherwise cancers would not develop following *H. pylori* eradication (28, 82) (S50). The *cagA*-transgenic mouse model provides evidence for the gastro-specific hyperproliferative effects of CagA; these lead to cancer-like changes in this model but more likely contribute to atrophy or metaplasia in humans. In Mongolian gerbils, specific rodent-adapted *cagA*⁺ *H. pylori* strains cause very rapid cancer-like pathology without atrophy, accompanied by activation of β -catenin (83), important in other mucosal cancers. Whether the lesions are analogous to or informative about human intestinal-type gastric cancer remains unclear; in humans, β -catenin activation is more common (and almost invariably found) in benign fundic gland polyps (S51). In total, these data indicate limitations in current animal models of human gastric carcinogenesis.

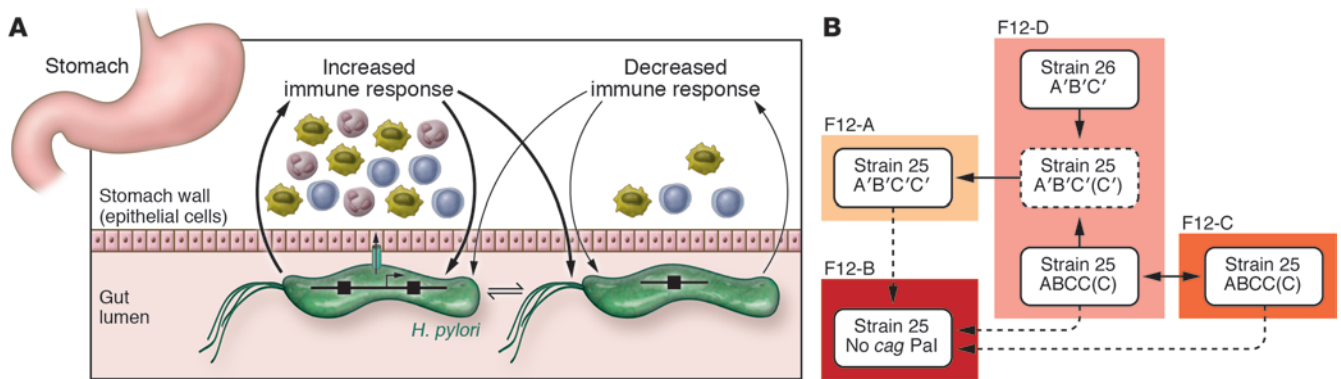


Figure 4 Variation of interactive factors. (A) Model of alternative host interactions according to status of mutable loci, modeled on the *cag Pal*. The *H. pylori* genome contains multiple hypermutable loci, creating many contingency genes. The status of each gene confers a particular phenotype to the *H. pylori* cell, affecting its host interactions. The strain on the left has an operating genetic element, which induces a greater host response; on the right is the same strain with a deleted element, inducing a lower response. The host responses create environmental constraints that select within the *H. pylori* population, creating a dynamic equilibrium (56). (B) Microevolution of *cagA* within a family. All four subjects in family F12 are colonized with strain 25, and subject D is also colonized with strain 26. Subjects C and D both have two clones of strain 25 with *cagA* with four and five type C (active) TPMs – these have evolved by simple duplication of the TPM-containing region. They are identical between these subjects, and so likely have been passed between them. Strain 25 in subject A possesses *cagA* with a variable region (VR) that is nearly identical to strain 26, implying these strains have recombined in subject D: strain 25 has acquired *cagA* from strain 26 and then been passed to subject A. Duplication of the TPM-C motif may have occurred within subject D or subject A. Strain 25 from subject B lacks the entire *cag Pal* and may have evolved by deletion from strain 25 from any family member. Solid lines indicate identical strains between hosts, whereas dashed lines represent proposed transmission. Adapted with permission from *Clinical cancer research* (43) (B).

Other *H. pylori*-associated diseases: under-recognized and under-researched

H. pylori may play a role in, or be a risk factor for, diseases other than gastric adenocarcinoma and peptic ulceration, some of which are important in global terms. Such diseases raise interesting and largely unanswered questions regarding disease-burden and/or pathogenesis. The strongest evidence for a causative role of *H. pylori* is in gastric lymphomas, especially low-grade B cell lymphomas of mucosa-associated lymphoid tissue (84) (S52). These tumors frequently regress following treatment to eradicate *H. pylori* (85) (S53), which has become first-line management in most situations. Studies also support a causative role for *H. pylori* in some cases of vitamin B12 and iron deficiency (86, 87) (S54–S57). Indeed, gastric atrophy is well known to cause both these conditions: gastric atrophy causes loss of production of intrinsic factor, a protein that binds vitamin B12 and facilitates its absorption; and the loss of gastric acidity caused by gastric atrophy, also may reduce iron absorption. In some cases, treatment to eradicate *H. pylori* corrects iron deficiency, implying that effects are not only through gastric atrophy (88); reduced acidity due to gastric corpus inflammation may be partly to blame. Due to space constraints, we do not discuss here the controversial observational and interventional studies suggesting a role in chronic idiopathic (auto-immune) thrombocytopenia purpura (89) (S58) or the even more controversial questions of whether *H. pylori* may affect the risk of acquiring exogenous infections or be a causal risk factor for cardiac and cerebral thrombotic disease.

Absence of *H. pylori* as a risk factor for disease

Humans likely always have been colonized by *H. pylori* and, as discussed above, *H. pylori* causes both immediate and long-lasting physiologic and immunologic changes. In historical terms, *H. pylori* is part of the normal microbiota in humans. Thus, from an evolutionary

viewpoint, *H. pylori*-adapted physiology and immunology is normal, and physiologic changes arise from the absence of *H. pylori*, most likely for the first time in human history (Figure 6A). As human evolution has occurred in the presence of *H. pylori*, our genetic constitution may be based around its presence. Although homeostatic mechanisms probably allow some adaptation to its absence, it should be asked whether such absence contributes to specific diseases, particularly those that have arisen in developed countries over the same time frame that *H. pylori* prevalence has fallen.

Absence of H. pylori causes hyperchlorhydria, and hence worsens acid-related esophageal diseases. Antral-predominant *H. pylori*-associated inflammation causes hyperchlorhydria, and corpus-predominant or pan-gastric inflammation cause atrophy and hypochlorhydria (1, 90) (S59, S60). The latter is more common and likely ancient; as such it should be considered “normal” for humans. Thus, *H. pylori*-negative persons (from an evolutionary viewpoint) have abnormally high acid output. If these persons also have a hiatus hernia, a lax lower esophageal sphincter, or increased transient lower esophageal sphincter relaxations (the proportion of the population that has this condition has increased in modern societies due in part to weight gain), gastroesophageal refluxate will be abnormally acidic, leading to more severe esophageal damage than seen in most *H. pylori* carriers.

Human data fit well with this hypothesis (S61). As *H. pylori* prevalence has fallen in developed countries, the prevalence of complications of gastroesophageal reflux (including esophagitis, Barrett esophagus, and esophageal adenocarcinoma) have increased (91, 92). Many cross-sectional studies show an inverse association between *H. pylori* status and severity of reflux symptoms and reflux esophagitis and an even more strong inverse association with prevalence of Barrett esophagus and esophageal adenocarcinoma (93–96). The strongest inverse associations are in East Asian populations (93) (S62). This is to be expected, as *H. pylori*-positive persons in

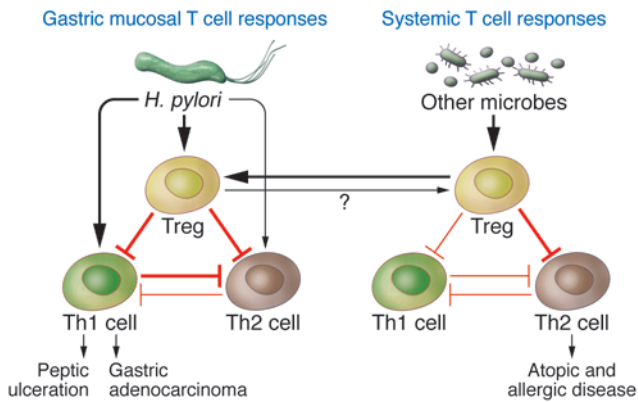


Figure 5

Th subsets in *H. pylori*-associated health and disease. *H. pylori* colonization is associated with strong Th1 and Treg responses. We speculate that historically the Treg response has been sufficient to downregulate the local gastric Th1 response thereby avoiding excessive gastric inflammation and gastroduodenal disease. Tregs are also induced by other microbes and through bystander effects may downregulate the *H. pylori*-associated Th1 response and disease. A low level of gastric Tregs is associated with an increased risk of peptic ulceration. We speculate that pre-19th-century humans had healthy levels of Tregs and thus that *H. pylori*-associated diseases (particularly peptic ulceration) were unusual. Either of two hypotheses could causally explain the rise in atopic and allergic disease with the disappearance of *H. pylori*. In the first hypothesis, loss of other infections common in childhood has led to reduced Tregs and thus to loss of Th2 suppression and increased Th2 diseases. Over the same time frame, the loss of Th1 suppression has led to the rise in *H. pylori*-associated diseases. In modern life, *H. pylori* is a marker for other childhood infections and a strong Treg response, explaining the negative association between *H. pylori* and diseases such as asthma. In the second hypothesis, loss of *H. pylori* itself has led to reduced Treg populations and a subsequent increase in Th2 responses; this could only be the case if *H. pylori*-associated Tregs had a systemic effect, which now has been observed. These hypotheses are not mutually exclusive.

those populations invariably have highly interactive strains (*cagA*⁺) and pan- or corpus-predominant gastritis and thus are expected to have low acid production and to be relatively protected against esophageal diseases. In developed countries, hosts with interactive (e.g., *cagA*⁺) strains (97, 98) (S63) and/or proinflammatory *IL1B* polymorphisms (98) (S64) (which are associated with corpus gastritis and hypochlorhydria) are least likely to have these conditions.

Clinical studies also are consistent and supportive. *H. pylori* eradication in patients with antral gastritis reduces acid production, whereas in patients with corpus gastritis, it usually increases acid production (99, 100). In East Asia, where pan- or corpus-predominant gastritis is common, *H. pylori* eradication is associated with increased GERD symptoms and esophagitis (101) (S65, S66); in Western countries, the response of GERD symptoms to *H. pylori* eradication is mixed, as would be expected in a population with mixed patterns of gastritis (102–104) (S67). Furthermore, reflux esophagitis following *H. pylori* eradication occurs particularly in individuals with hiatus hernias or weak lower esophageal sphincters (101) (S65, S66). Taken together, these studies suggest that although reflux esophagitis and its sequelae may be caused by abnormalities of the lower esophageal sphincter, on average, they are considerably worsened by the hyperchlorhydria of *H. pylori*-free humans.

Absence of H. pylori causes hyperghrelinemia and reduced gastric leptin: does this contribute to obesity? Associations between *H. pylori* and BMI have biologic plausibility. *H. pylori* colonization has specific effects on the hormones leptin and ghrelin, which are produced in the stomach. Leptin is a major inhibitor of appetite and a long-term controller of fat stores (S68, S69). Although *H. pylori* affects expression of gastric leptin, no substantial effects on serum leptin have been detected (105, 106), probably because the stomach is a minor producer of leptin compared with fat stores. However, the gastric corpus also produces two-thirds of the body's ghrelin (107) (S70). Ghrelin is thought to signal immediate hunger and appetite rather than have a role in the long-term control of body habitus (107). However, ghrelin administration to rodents increases body weight, and ghrelin antagonism reduces it (S71). *H. pylori*-positive persons have lower levels of ghrelin-producing cells, gastric ghrelin production, and serum ghrelin (108) (S72, S73), and *H. pylori* eradication increases ghrelin levels (106). Ghrelin levels are more profoundly reduced in persons colonized with more interactive (*cag*⁺) strains of *H. pylori* (109). However, effects on ghrelin are seen only when the gastric corpus is inflamed, not in antral-predominant gastritis (110).

Several lines of evidence suggest an association between *H. pylori* colonization and body habitus in children and adults. Longitudinal studies provide evidence that growth of children slows at the time of *H. pylori* acquisition and does not subsequently completely catch up to that in children not colonized with the organism (111, 112). Cross-sectional studies show that *H. pylori*-positive children tend to be shorter and lighter than their noncolonized contemporaries (113) (S74). *H. pylori*-positive adults in East Asia tend to have a lower BMI than those who are not colonized with the bacterium, and several East Asian studies have shown that *H. pylori* eradication leads to weight gain in adults (105, 114). However, in a very large, population-based cohort from the USA, no association was demonstrated between *H. pylori* status and BMI (115, 116). There is no direct evidence that *H. pylori*-induced changes in leptin or ghrelin causally explain these associations, but the data are consistent with such a hypothesis. For example, the lower BMI in *H. pylori*-positive adult East Asians, who invariably carry the more interactive *cag*⁺ strains and have pan-gastritis, is consistent with the lower ghrelin levels observed in individuals who carry these strains (109, 110) and in those who have a pan- or corpus-predominant gastritis (110).

Because the rise in obesity and metabolic diseases, including type 2 diabetes, have a similar time frame to the disappearance of *H. pylori*, could they in part be caused by this disappearance (S75)? Certainly the high gastric and serum ghrelin (108) (S72, S73) and the low gastric leptin (105) seen in the absence of *H. pylori* provide a biologically plausible link. However, large population-based datasets show no association between *H. pylori* and obesity (115, 116). One possibility is that, as with reflux esophagitis, data from Western countries obscure the complexity of the issue, because individuals may have antral or corpus gastritis and strains of high or low interactivity. This variation would lead to a constellation of states of ghrelin regulation. A second possibility is that the ready availability of food in Western culture may obliterate other effects. In East Asian populations, where most people have corpus gastritis and highly interactive strains of *H. pylori*, obesity has been associated with *H. pylori*-negativity (117). The hypothesis that modern obesity is due in part to absence of *H. pylori*, and that the mechanism is through disturbances in gastric hormones, remains unproven but deserves more extensive investigation.

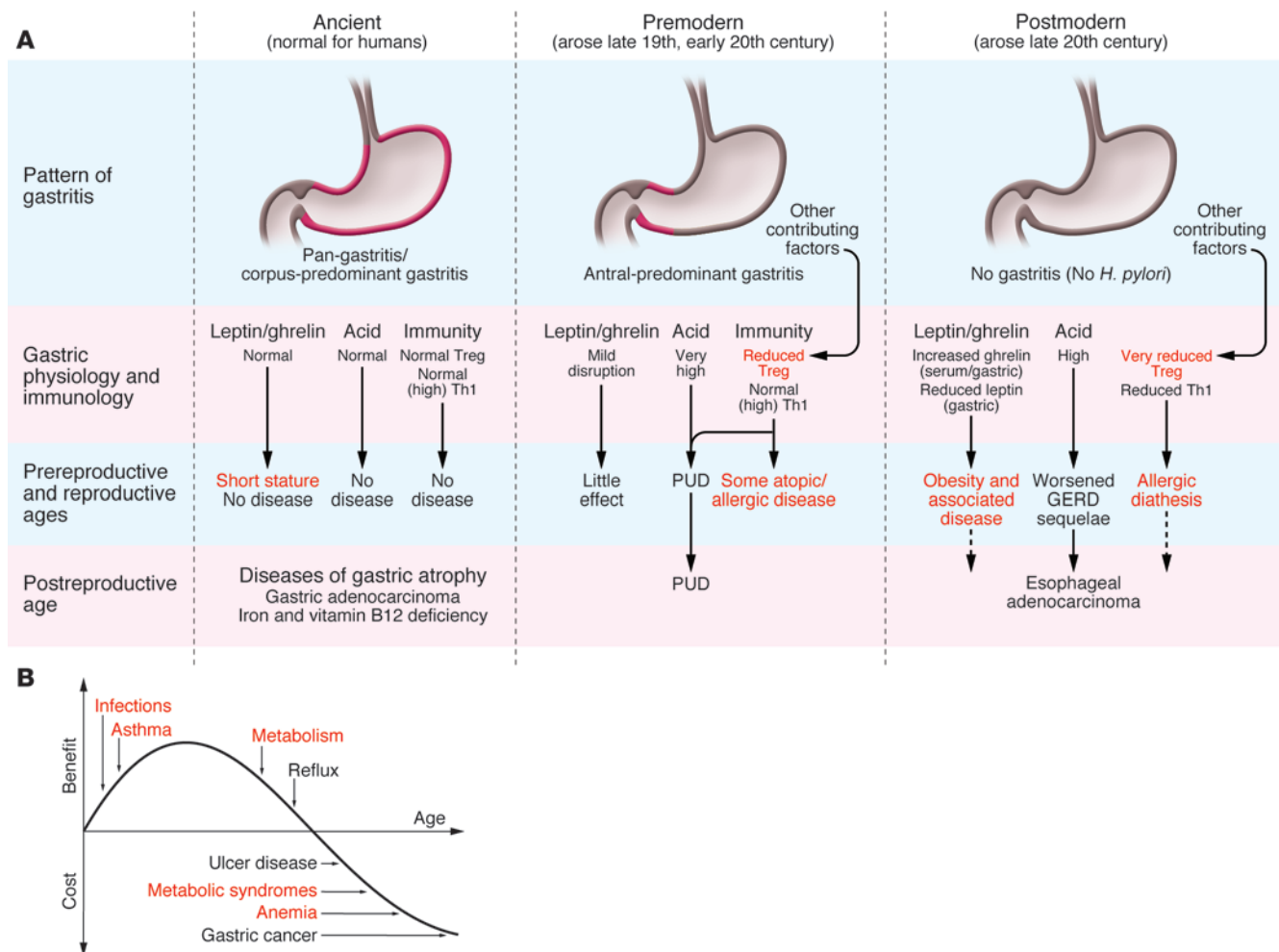


Figure 6 Overviews of *H. pylori* relationships to health and disease. (A) Ancient, premodern, and postmodern stomachs. From ancient times, normal human physiology in the presence of *H. pylori* pan-gastritis has avoided disease until the gastric cancers of old age. We speculate that premodern changes in the pattern of colonization and inflammation in the stomach resulted in changes in physiology and the rise of peptic ulcer disease (PUD). In the postmodern (current) era, the absence of *H. pylori* leads to distorted physiology, to which we have not fully adapted. This may have led to disease in some children and adults but avoids gastric cancer in old age. (B) A model of the proposed biphasic nature of *H. pylori* and human disease. Because we have coevolved with *H. pylori*, the changed physiology resulting from an *H. pylori*-free stomach may contribute to some modern diseases. Thus, from a postmodern viewpoint, *H. pylori* may confer benefits to humans early in their life span. Possible examples include reducing infectious diseases, controlling allergy, regulating gastric hormones such as leptin and ghrelin (benefits uncertain), and reducing gastroesophageal reflux disease sequelae. Later in life, *H. pylori* has biological costs, inducing ulcers, (possible) metabolic disturbances, anemia, and gastric cancers, all more prominent in an ageing population. In both A and B, black text indicates information for which there is strong evidence — for these, the balance is toward a net deleterious effect of *H. pylori* on human health — and red text indicates information for which evidence remains under debate. *cagA*-negative strains are less interactive with humans, conferring smaller risks and putative benefits.

Absence of an immunologic response to H. pylori: an important contributor to atopic and allergic disorders? The hygiene hypothesis proposes that childhood exposure to infections aids maturation of immune responses that offer protection against atopic, allergic, and autoimmune diseases (S76). A corollary is the disappearing microbiota hypothesis, in which the protective agents are endogenous organisms that have long been present in humans but are disappearing (118) (S75). *H. pylori* would be a logical contributor to this hypothesis and could even be a dominant player. The bacteria colonize young children, and the systemic effect is sufficient to disturb growth (111, 112). *H. pylori* induces inflammation in a large mucosal organ and importantly persists throughout life, so effects are not transient.

Cross-sectional epidemiologic studies show negative associations between *H. pylori* and various atopic and allergic diseases (119–121) (S77, S78). The problem with such studies is that *H. pylori* positivity is associated with more crowded living conditions and poor hygiene in childhood (S79). Since these factors also are associated with other childhood infections, *H. pylori* status may merely be a marker for these. However, the association with childhood asthma is stronger for *cagA*⁺ strains of *H. pylori* (119), and there is no evidence that *cagA*⁺ strains are a better marker of other childhood infections than are *cagA*⁻ strains.

Plausible mechanisms exist that could explain a causal negative association between *H. pylori* and atopy/allergy. Atopic and aller-



gic diseases are characterized by predominant Th2 effects, and *H. pylori* is associated with a gastric Th1 response. One possibility is that *H. pylori* drives a more generalized Th1 response that suppresses Th2 effects, but there is little evidence for this. More plausibly, as we and colleagues have previously suggested, the high levels of Tregs associated with *H. pylori* colonization may contribute to prevention of allergic diseases, and *H. pylori*-free humans are thus more prone to these diseases (S36, S80). In support of this, *H. pylori*-positive people have higher levels of gastric Tregs than those without the organism (70, 72) (S43). More importantly, the increase in Tregs is not confined to the gastric mucosa; circulating Tregs also are increased in number (122) (S81). In addition, in mice experimentally infected with *H. pylori*, systemic Tregs are increased and these suppress other immune responses, one effect of which is to facilitate *H. pylori* colonization (123). The excess Tregs may have immunosuppressive activity in humans as well: among *H. pylori*-positive persons, those with fewer Tregs are more likely to have peptic ulcers (70) and so presumably have more intense gastritis. Finally, in *cagA*⁺ *H. pylori* colonization, mucosal Tregs may be more numerous and mucosal levels of the immunomodulatory cytokine IL-10 may be higher than in *cagA*⁻ colonization (124). If the same phenomenon applied to circulating Tregs, it could potentially explain the stronger, negative association with childhood asthma of *cagA*⁺ strains (119). Taken together, these studies imply a plausible link between *H. pylori*, Tregs, and reduction in risk of allergic and autoimmune disease. Interventional and mechanistic studies in relevant animal models and in humans are needed to test the hypothesis.

Conclusions

H. pylori and humans have coevolved for at least for 50,000 years and probably for much longer. As such, *H. pylori* colonization has been essentially universal, and the usual pattern of inflammation has likely been pan-gastric. This historically “normal” association enhances risk of gastric cancer, a disease known since Hippocrates. Since gastric cancer nearly exclusively occurs after the main repro-

ductive years, there has been no substantial selection against it; indeed, we have suggested that cancers may have been beneficial to premodern societies by causing the demise of elderly individuals in an age-dependent manner (1). In contrast, peptic ulceration is a modern disease, likely due to recent environmental disruptions of our relationship with *H. pylori*. The extent to which other *H. pylori*-associated diseases are ancient or modern is undetermined.

Absence of *H. pylori* from human populations is new. Our physiology and immunology have evolved in the presence of persistent gastric *H. pylori* colonization and so are disrupted by its absence. In the case of acid homeostasis, this disruption probably contributes to modern disease, specifically the severity of reflux esophagitis and the consequent esophageal adenocarcinoma, which is increasing dramatically in incidence (92). We and others have hypothesized that absence of *H. pylori* also may contribute to other modern diseases, including obesity and allergic disorders. From an evolutionary viewpoint, *H. pylori* may be neither beneficial nor harmful. However, from a 21st century view of human health, the perspective differs. If confirmed, the recent data imply a model of early life benefit from *H. pylori* and late-in-life cost (Figure 6B).

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