



Helicobacter pylori persistence: biology and disease

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Helicobacter pylori are bacteria that have coevolved with humans to be transmitted from person to person and to persistently colonize the stomach. Their population structure is a model for the ecology of the indigenous microbiota. A well-choreographed equilibrium between bacterial effectors and host responses permits microbial persistence and health of the host but confers risk of serious diseases, including peptic ulceration and gastric neoplasia.

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The twin hallmarks of the interaction between *Helicobacter pylori* and humans are its persistence during the life of the host, and the host's responses to its continuing presence. This conflict appears paradoxical, but both the microbes and the host adapt to the other in the form of a long-standing dynamic equilibrium (1, S1). Our understanding of the phenomena underlying these interactions is growing. The relationships are important, both because of the major role of *H. pylori* in promoting risk of peptic ulcer disease (2) and non-cardia adenocarcinoma of the stomach (3), and because of the emerging evidence that gastric *H. pylori* colonization has a protective role in relation to severe gastro-esophageal reflux disease and its sequelae, Barrett esophagus and adenocarcinoma of the esophagus (reviewed in ref. 4). New studies suggest other important impacts of *H. pylori* colonization on human physiology (5, 6).

We now present a general model for this host-microbial interaction and then turn to examples of specific operating mechanisms. Although *H. pylori* is unique in colonizing the human stomach, the principles governing the interaction are paradigms for understanding both commensalism and long-term parasitism. Such insights aid our understanding of

disease processes as diverse as chronic inflammation, oncogenesis, and hormonal dysregulation and may be relevant to modern epidemic problems such as obesity and diabetes.

A general model of host-microbial persistence

Much evidence indicates that *Helicobacter* species are the indigenous biota of mammalian stomachs, and that *H. pylori* is the human-specific inhabitant (Figure 1a), having been present for at least tens of thousands of years, and probably for considerably longer (7–9). Therefore, coevolution of microbe and host might be expected, and for *H. pylori*, substantial evidence supports this notion (10), with important implications.

Microbial colonization of a host locale affects the surrounding tissue through the occupation of niches, utilization of resources, and excretion, all of which may be considered as signals to the host (Figure 1b). The host also signals the microbe in the form of pressure, temperature, and chemical milieu (including host-defense molecules). Although these signals could be uncoordinated, coevolution implies linkage, in which the signals of one party affect the signals of the other (Figure 1c). Microbial persistence requires equilibrium, which only can occur when negative feedback is present (1, S1). This simple model forms the basis for understanding *H. pylori* persistence, and microbial persistence in general. If the microbial population includes differing strains, as clearly occurs for *H. pylori* (11, S2, S3), then the host signals are selective forces (Figure 1c), as it is this selected microbial population rather than the individual cell that is the host-signaling entity (Figure 1d). Many bacterial populations are not entirely clonal, reflecting both point mutations and recombination; *H. pylori* is a particularly extreme example with both a high mutation rate and a very high

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Nonstandard abbreviations used: C-terminal Src kinase (Csk); G protein-coupled receptor kinase-interactor 1 (Git1); Toll-like receptor (TLR).

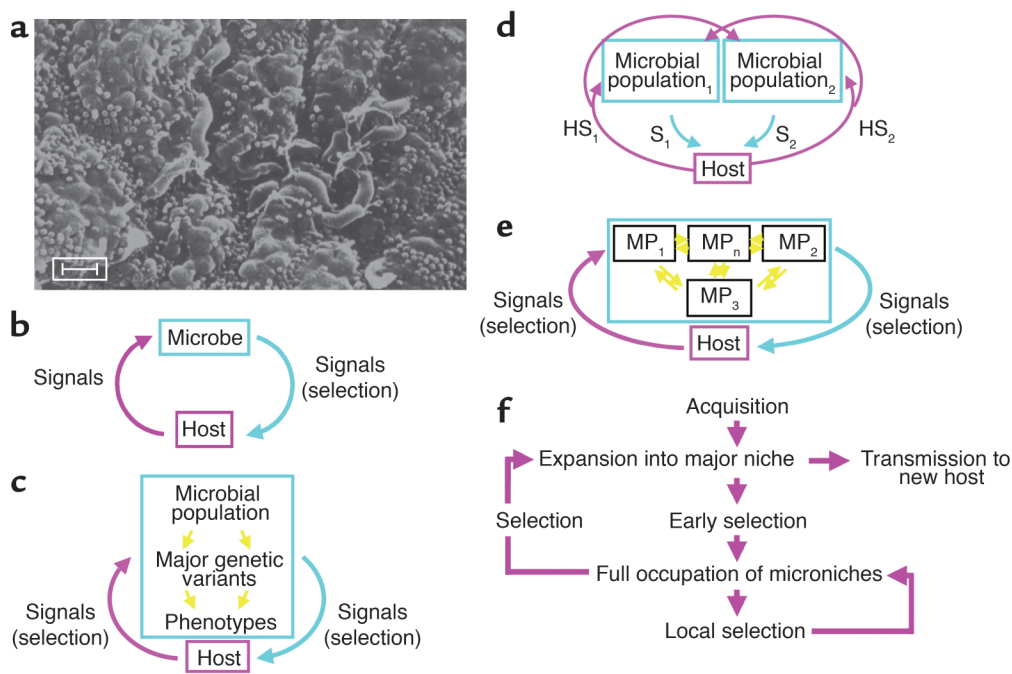


Figure 1

Models of the cross-signaling between obligate parasites and their hosts. (a) *H. pylori* in the gastric (antral) niche, inhabiting the luminal mucus layer overlaying the epithelium. Scale bar: 1 μm . (b) Model 1: A coevolved microbe sends chemical and physical (contact) signals to its host. The host's signals, including motion, temperature, and chemicals (including host-defense molecules), affect microbial growth. In a local niche, equilibrium can be reached if there is negative feedback in the cross-signaling (1, S₁). The microbial signals may alter selection of growing host-cell populations (e.g., by antiapoptotic mechanisms). (c) Model 2: The microbial population includes genetic variants. Now, the host signals select for different bacterial phenotypes, and thus genotypes. (d) Model 3: Due to ongoing variation and selection, each microbe becomes a microbial population of related variants. The signals of each variant affect not only the local host signals, but also those affecting other microbial populations. This is represented by two distinct microbial populations, with each signaling the host (S₁, S₂) and inducing specific host signals (HS₁, HS₂). Increasing numbers of populations markedly and nonlinearly increase the complexity. (e) Model 4: The microbial populations are not clonal but can exchange genetic information; host selection, based on microbial genes or gene fragments rather than cells, determines the (dynamic) equilibria between the microbial populations (MP_n). For the naturally competent, extensively recombining *H. pylori* cells, model 4 best reflects the fluidity of population structures during persistent colonization (11, S₂, S₃). (f) Schematic of adaptation to individual hosts. After *H. pylori* acquisition and expansion into a major niche, early selection allows occupation of microniches, where further local selection determines predominant populations. Both local selection and global selection determine overall population structure, and the probability of transfer of particular genotypes to new hosts. Local resource differences and barriers to diffusion of bacterial cells allow establishment of distinct subpopulations. External fluctuations, such as incomplete antibiotic treatment, can markedly change genotype distribution.

recombinational frequency (12, 13). Thus, each host is colonized not by a single clone but rather by a cloud of usually closely related organisms (11), resembling the “quasispecies” observed with persistent RNA viruses, such as hepatitis C and HIV. This microbial variation affects the signals to the host; for example, within an *H. pylori* population, individual cells may or may not express specific host-interaction molecules (e.g., CagA) that affect host biology in a directed manner. Consequent host “signals,” ranging from increased nutrient supply through immune effectors to changes in the gastric microenvironment, are differentially selective for specific *H. pylori* genes. Thus, each host is colonized by a fluid bacterial gene pool, with genotype dominance determined by selection (Figure 1e). In sum, concepts of such highly plastic populations subject to host-specific selection provide models to explain the facility of *H.*

pylori to persist, the presence of different strains as well as variants of these strains in individual hosts, and the ability of *H. pylori* to colonize essentially all humans (Figure 1f), despite our heterogeneity.

***H. pylori* adaptations that facilitate persistence**

H. pylori mechanisms to increase diversity. The remarkable diversity of *H. pylori* (12, S₄) may be viewed as evidence of a versatile population, able to maximize resource utilization in a variety of niches and microniches and to avoid host constraints. Such constraints include not only host immunity but also developmental changes in the gastric epithelium, acidity, and nutrient availability. Generation of diversity typically involves endogenous (point) mutations, and recombination; *H. pylori* has mechanisms for each (Table 1). Mutation rates are not constant in bacterial populations but subject to environmental signals, and with-

Table 1
H. pylori mechanisms to increase diversity

Mechanism	Example	Reference
Endogenous mutation	Mutator phenotype	13
Intragenomic recombination	Nonrandom, extensive repetitive DNA	16
	Lack of mismatch-repair systems	19
	Active gene conversion	20
Intergenomic recombination	Natural competence	S6, S7
Niche sectoring	Strain-specific restriction	21, S10, S12
	Ligand specificity	22, 23, S13

in large populations a small proportion of cells arise that have heightened mutation rates (S5). For *H. pylori*, most strains would be considered to have this hypermutator phenotype (13), which favors the emergence of variants after selective pressure. A good example of efficient adaptive point mutation by *H. pylori* is the rapid development in the bacterial population of high-level resistance to commonly used antibiotics, such as the macrolide clarithromycin (14).

H. pylori cells are also highly competent for uptake of DNA from other *H. pylori* strains (S6, S7, S8). Analysis of *H. pylori* sequences shows strong evidence of recombination between strains, to the degree that clonal lineages are largely obscured (12, 15). Substantial intragenomic recombination occurs, based largely on the presence of repetitive DNA sequences (16, 17). Repetitive DNA allows for high-frequency deletion and duplication, including slipped-strand mispairing (18, S9). However, because *H. pylori* is naturally competent, any genetic element that is lost may be regained from either unaffected sectors of the population of that strain or from another strain (17, S10). A lack of mismatch-repair systems (19) may increase the frequency of random variation, but it also may facilitate gene conversion, which minimizes genomic diversity of those alleles present in multiple copies (20). Thus, *H. pylori* can maximize diversity of sequences under strong selective pressure, while maintaining alleles that are critical for its lifestyle.

Introduction of a new *H. pylori* strain into an already colonized host increases the total diversity in the population, since each resembles a quasispecies; however, transformation of one strain by the other reduces diversity. All *H. pylori* strains contain multiple restriction-modification systems, but rarely do any two have the same complement (21, S11). Thus, there are restriction barriers to transformation, a property that may maximize coexistence of parallel gene pools, by slowing genetic exchange (S12). Local selection also may add to genetic diversity within an individual stomach; separate gastric microniches are likely colonized by subpopulations that have particular attributes to maximize fitness, for example, ligand specificity for local receptors (22, 23, S13).

Human interaction domain 1: cag island. In 1989, a strain-specific *H. pylori* gene, *cagA*, was identified (24), which now has been recognized as a marker for strains that confer increased risk for peptic ulcer disease (25, 26) and gastric cancer (27, S14). No homologs are known for *cagA* in other *Helicobacter* species or in other bacteria, suggesting that it reflects a human gastric-specific gene. *cagA* (S15, S16) is a marker for the 35- to 40-kb *cag* (pathogenicity) island that is flanked by 39-bp direct DNA repeats (S17, S18). Strains without the island possess a single copy of the 39-bp sequence, in a conserved gene (glutamate racemase [*glr*]), and through transformation the entire island may be restored or lost (28). *H. pylori* strains with partial *cag* islands also have been identified, and variation in island size and genotype within individual hosts is well described (S3, S19, S20).

The island contains genes encoding a type IV secretion system, which in other bacteria inject macromolecules (i.e., DNA and proteins, such as pertussis toxin) into host cells (29). One substrate for the type IV system in *H. pylori* is the *cagA* product (30, 31, S21–S23), which is injected into epithelial cells, both in vitro (30, 31, S22, S23) and in vivo (32) (Figure 2). In many strains, the CagA protein contains tyrosine-phosphorylation sites (30, 31, 33, S21–S23) that are recognized by the host cell Src kinase (34). Once phosphorylated, CagA interacts with SHP-2, a tyrosine phosphatase (35), which affects spreading, migration, and adhesion of epithelial cells (32). This phenomenon can be assessed in vitro by a change in epithelial cell morphology to the scattered, or “hummingbird,” phenotype (31).

The injected CagA protein also interacts with Grb2 and activates the Ras/MEK/ERK pathway, leading to the phenotypes of cell scattering (in AGS cells) and proliferation (in MDCK cells) (36). Tyrosine-phosphorylated CagA binds and activates C-terminal Src kinase (Csk) via its SH2 domain, which in turn inactivates the Src family of protein-tyrosine kinases. Since this signaling may induce apoptosis, the Csk pathway may attenuate the other CagA interactions (37). By inactivating Src, tyrosine-phosphorylated CagA induces dephosphorylation of cortactin, which then colocalizes with filamentous actin (F-actin), in the tip and base of hummingbird protrusions (38). Thus, the *H. pylori* CagA protein interacts with several of the major signal-transduction pathways present in epithelial cells. *H. pylori* cells with the *cag* island deleted have remarkably little interaction with AGS cells in tissue culture (39); conversely, the *cag* apparatus promotes antiapoptotic pathways, which may aid persistence by slowing turnover of the epithelial cells to which they are attached.

There is extensive *H. pylori* variation in this major interactive modality; clonality and the lack thereof each imply important, albeit different, selective pressures. In individual strains, parts of the *cag* island, including *cagA*, may be deleted (S3, S19, S20); *cagA*

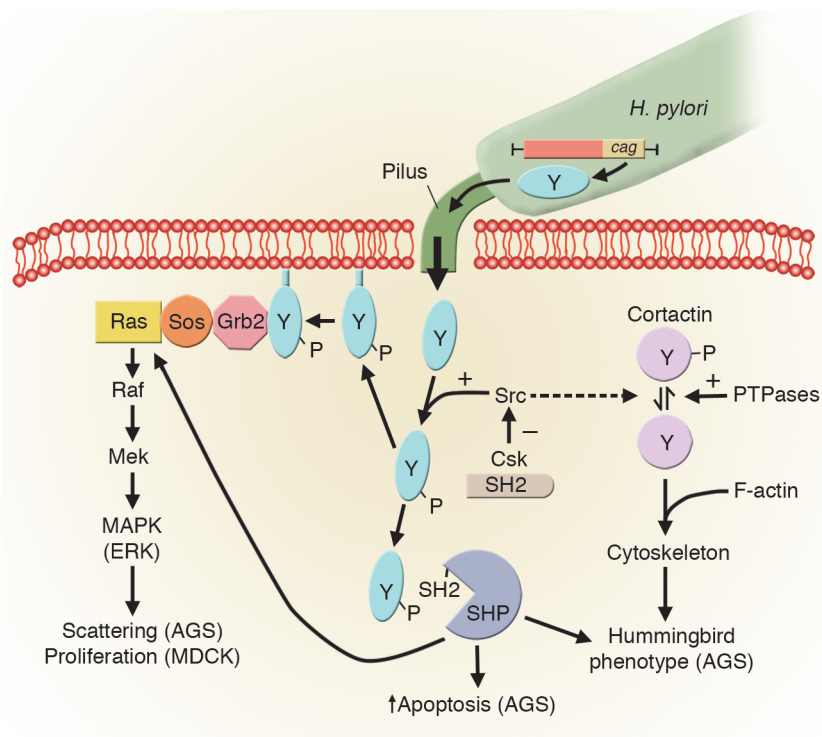


Figure 2
CagA interaction with epithelial cells. *H. pylori* cells with intact *cag* islands, including an active type IV secretion system, possess a pilus composed of CagY protein. The *cagA* product is injected into the cytoplasm of the host cell, where tyrosine (Y) residues near its COOH-terminus are phosphorylated. Phosphotyrosine-CagA interacts with several major signal-transduction pathways in the host cell (40, S113), affecting phenotypes including cell morphology, proliferation, and apoptosis (see text). ERK, extracellular signal-regulated kinase; PTPase, protein-tyrosine phosphatase; P, phosphate.

itself shows phylogeographic variation with Eastern, Western, and hybrid genotypes (S24). The DNA sequences encoding the tyrosine-phosphorylation motifs are variable in number and flanked by repetitive elements, allowing their deletion or duplication, which affects the phenotype of the injected CagA protein (33). Thus, *H. pylori* populations possess extensive repertoires that permit variation of Cag phenotypes in response to particular hosts, micro-niches within these hosts, or changing environmental circumstances. Nevertheless, antibody responses to CagA remain relatively constant over at least 20 years (40) in an individual host, implying an overall stability in the interactive relationship, best represented in Figure 1e.

Human interaction domain 2: vacA. Culture supernatants from some *H. pylori* strains release a high-molecular weight multimeric pore-forming protein, VacA, that causes massive vacuolation in epithelial cell lines (41, S25). As with *cagA*, no close homologs of *vacA* exist in other *Helicobacter* species or in other bacteria (42, S26–S28), which suggests its importance in the specific relationship of *H. pylori* with the human stomach. However, unlike *cagA*, *vacA* is conserved among all *H. pylori* strains, although significant polymorphism exists (43). *vacA* alleles possess one of two types of signal region, s1 or s2, and one of two mid-regions, m1 or m2, occurring in all possible combinations (Figure 3). Research has focused on the most interactive (vacuolating) type, s1/m1.

VacA has several specific effects that may contribute to *H. pylori* persistence in the gastric niche. Firstly, it forms pores in epithelial cell membranes, allowing egress of anions and urea (44, 45, S29, S30). This is

important since urea hydrolysis, catalyzed by *H. pylori* urease, protects against gastric acidity (S31). VacA also induces loosening of epithelial tight junctions, potentially allowing nutrients to cross the mucosal barrier to *H. pylori*'s gastric luminal niche (46, S32). Recent work in vitro suggests that VacA may help *H. pylori* persistence by specific immune suppression. VacA blocks phagosome maturation in macrophages (47), selectively inhibits antigen presentation in T cells (48, S33), blocks T cell proliferation, and downregulates Th1 effects by interacting with calcineurin to block signaling (49). Besides these actions that may benefit *H. pylori* persistence, VacA also has direct cell-damaging effects in vitro, inducing cytoskeletal changes, apoptosis, and suppression of epithelial cell proliferation and migration (50–52, S34, S35), as well as cell vacuolation. Whether these effects are germane in vivo is unknown, but cell damage could aid nutrient delivery from the gastric mucosa.

Which in vitro effects of VacA are most important for *H. pylori* persistence in vivo is unclear, and animal models have not clarified this. In piglets, gerbils, and mice, VacA-null strains persist without apparent disadvantage (S36–S38), although in competition experiments in mice, VacA-null mutants colonize less well than their VacA⁺ wild-type parents (S38). However, animal models have proved useful for characterizing the damaging effects of VacA. Although VacA is not necessary for gastric ulcer formation, in *H. pylori*-colonized Mongolian gerbils its presence increases the risk (53). Mice administered VacA orally develop gastric ulcers (54, S28), but mice deficient in the protein tyrosine phosphatase receptor type Z, polypeptide 1 (*Ptprz*^{-/-} mice) do not (54). *Ptprz* is one of several puta-

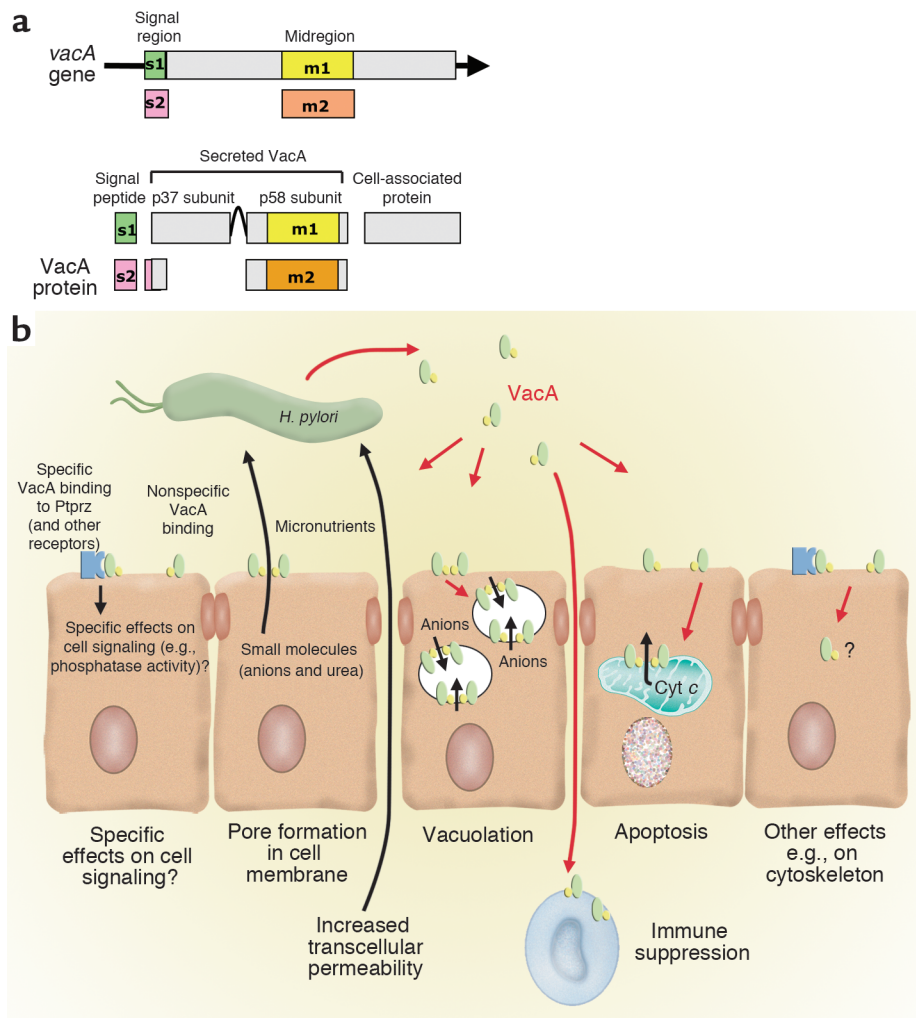


Figure 3

VacA polymorphism and function. (a) VacA polymorphism. The gene, *vacA*, is a polymorphic mosaic with two possible signal regions, s1 and s2, and two possible mid-regions, m1 and m2. The translated protein is an autotransporter with N- and C-terminal processing during bacterial secretion. The s1 signal region is fully active, but the s2 region encodes a protein with a different signal-peptide cleavage site, resulting in a short N-terminal extension to the mature toxin that blocks vacuolating activity and attenuates pore-forming activity. The mid-region encodes a cell-binding site, but the m2 type binds to and vacuolates fewer cell lines in vitro. (b) VacA biological activities. Secreted VacA forms monomers and oligomers; the monomeric form binds to epithelial cells both nonspecifically and through specific receptor binding, for example, to Ptpzr, which may modulate cell signaling. Membrane-bound VacA forms pores. Following VacA endocytosis, large vacuoles form, but, although marked in vitro, these are rarely seen in vivo. VacA also induces apoptosis, in part by forming pores in mitochondrial membranes, allowing cytochrome *c* (Cyt *c*) egress. Although the presence of cytoplasmic VacA is implied rather than demonstrated, yeast two-hybrid experiments show potential for specific binding to cytosolic targets including cytoskeletal proteins, consistent with observed cytoskeletal effects. Finally, VacA has suppressive effects on immune cell function. The relative importance of these effects on *H. pylori* persistence and host interaction remains unclear.

tive VacA cellular receptors, and VacA-induced activation increases tyrosine phosphorylation of G protein-coupled receptor kinase-interactor 1 (Git1), leading ultimately to epithelial cell detachment (54). VacA also may have important effects on nonepithelial cells: in rats, only VacA⁺ strains induce macromolecular leakage from the gastric microcirculation (S39).

H. pylori strains with different forms of *vacA* exhibit varied phenotypes and have particular associations with gastro-duodenal diseases. The *vacA* signal region encodes the signal peptide and the N-terminus of the

processed VacA toxin: type s1 VacA is fully active, but type s2 has a short N-terminus extension that blocks vacuole formation (55, 56) and attenuates pore formation in eukaryotic membranes (S40). *vacA* s2 strains are rarely isolated from patients with peptic ulcers or gastric adenocarcinoma (43, 57, S41, S42). The *vacA* mid-region encodes part of the toxin cell binding domain. s1/m2 forms of VacA bind to and vacuolate a narrower range of cells than s1/m1 forms and induce less damage, yet they also act as efficient membrane pores and increase paracellular permeability (56, S30,

S32, S43). *vacA* s1/m1 strains are most closely associated with gastric carcinoma (58, 59, S44). Natural persistence of distinct polymorphic forms of *vacA* in diverse human populations implies that each offers a survival advantage. That particular forms of VacA potentially induce different levels of *H. pylori*-host interaction fits well with the general model in which less interactive strains cause diminished tissue injury and disease, and highly interactive strains, while benefiting from their interaction, are more likely to affect their niche and thus injure their host.

Interaction between the interacting domains. The *cag* island and *vacA* are far apart on the *H. pylori* chromosome (19, 60), yet there is a strong statistical linkage between the s1 genotype of *vacA* and the presence of the *cag* island (43, S24); similarly, the s2 genotype is associated with lack of the *cag* island (43). Although these phenomena may reflect founder effects, the panmixis of *H. pylori* (12) suggests selection for the skewed relationships. This is not a simple interdependence of function: in a *cag*⁺/*vacA* s1/m1 strain, *cag* mutagenesis does not abolish vacuolating cytotoxin activity, nor does disruption of *vacA* abolish the *cag*⁺ phenotype (29, 31, 39, S45, S46). However, there are subtle quantitative effects: *vacA* disruption slightly reduces early tyrosine phosphorylation of CagA during epithelial cell interaction, whereas, in contrast, *cag* disruption slightly increases VacA-induced vacuolation (61). One contributor to these effects may be the colocalization on lipid rafts of tyrosine-phosphorylated Git1/Cat1 (substrate molecules of the VacA receptor phosphatase Ptpz) and tyrosine-phosphorylated CagA (61).

The minor interactions between CagA-induced and VacA-induced effects on epithelial cells are unlikely to explain their close linkage in *H. pylori*. An alternative hypothesis is that VacA selects for a functional *cag* island, since VacA-induced immune suppression might not permit adequate nutrition of the *H. pylori* population (Figure 1). The effect of *cag*⁺ strains in weakening epithelial tight junctions (62) may enhance nutrient flow to the bacteria and allow better VacA delivery to the mucosa. For the less potent s2 strains, this selection would be less substantial and might be counterbalanced by the phenotypic costs of maintaining the *cag* island. Whatever the true selective benefits VacA and *cag* offer each other, in conditions with multiple strains colonizing individual hosts, each major genotypic combination (*cag*⁺/*vacA* s1 or *cag*⁻/*vacA* s2) could occupy a relatively exclusive physiological niche. Recombination events mixing the loci would be selected against. With diminishing multiplicities of *H. pylori* colonization (7), the strain (and genotype) diversity within a host would be reduced, diminishing the resource base for the overall population. Thus, once *H. pylori* transmission within a human population declines, the decline may accelerate because of diminished vitality of the colonizing bacterial populations in individual hosts.

Immune evasion and manipulation by H. pylori. If a microbe is to persist in a vertebrate host, its biggest challenge is to avoid clearance by the immune system. Transient *H. pylori* colonization has been documented in both primates and humans (63, 64, S47), implying that persistence does not inevitably follow acquisition. The race between *H. pylori* adaptation to a specific host (Figure 1) and the development of effective immunity also implies the feasibility of vaccine development. However, usually, following *H. pylori* acquisition, there is rapid host recognition in the form of both innate and acquired immune responses, including generation of specific local and systemic antibodies (65, S48–S51). Once chronicity is established, the immune stimulation appears remarkably constant; for example, antibody titers remain stable for over 20 years (40), consistent with a model of dynamic equilibrium (Figure 1). The ubiquity and duration of host recognition of *H. pylori* and yet the lifelong colonization by the bacterium demonstrate the effectiveness of *H. pylori*'s strategies to evade host immunity. The important first step is to survive without tissue invasion (Table 2), and the bulk of *H. pylori* cells, if not all of them (Figure 1a), reside in the gastric lumen (66, S52) beyond the reach of most host immune recognition and effector mechanisms (S48, S52, S53). However, even in this niche, some *H. pylori* cells establish intimate contact with the surface epithelium (S52, S54), some *H. pylori* proteins cross the epithelial barrier (67), and both innate and acquired immune systems are activated (65, S48–S50). Although it is not able to completely avoid immune activation, *H. pylori* has evolved mechanisms to reduce recognition by immune sensors, downregulate activation of immune cells, and escape immune effectors.

Innate immune system recognition of microorganisms involves Toll-like receptors (TLRs) that discriminate pathogen-associated molecular patterns (S55). TLR stimulation triggers proinflammatory signaling through NF-κB activation, and *H. pylori* has evolved to minimize such stimulation. TLR5 recognizes bacterial flagella such as those of *Salmonella typhimurium* but is not stimulated by *H. pylori* flagella (S56). TLR9 recognizes the largely unmethylated DNA of most bacteria (S57), but the highly methylated *H. pylori*

Table 2
Immune evasion by *H. pylori*

Mechanism	Example	Reference
Sequestration	Luminal colonization	66, S52
Minimization of innate immunity	Low toxicity of LPS DNA methylation	S58–S60 S11
Downregulation of immune effectors	VacA suppression of T cell responses Interference with phagocytosis	48, 49 76
Mimicry of host antigens	Lewis expression	74
Antigenic variation	CagY	75

DNA likely minimizes recognition (S11). *H. pylori* LPS is anergic compared with that of other enteric bacteria because of lipid A core modifications (S58–S61), and while it stimulates macrophage TLR4 (68, S61), it does not stimulate gastric epithelial TLR4 (69). Although *H. pylori* is relatively camouflaged from innate immune sensors on cell surfaces, *cag*⁺ strains do stimulate NF- κ B activation in epithelial cells (70, S62), apparently through recognition by Nod1 (S63), an innate intracellular pathogen-recognition molecule that detects soluble components of bacterial peptidoglycan (71). How such components are delivered to the epithelial cytoplasm by the *cag*-encoded type IV secretion system remains unclear, but the resultant NF- κ B-induced proinflammatory cytokine expression is an important and continuing stimulus to inflammatory cell infiltration and thus to pathogenesis (65, S49).

H. pylori also activates the acquired immune system, as indicated by both humoral and cellular recognition of its antigens (72, S48, S50, S53), although it has evolved to substantially downregulate and avoid acquired immune effectors. Recognition by the acquired immune system requires antigen presentation, and *H. pylori* interferes with both uptake and processing of antigens, partially through a VacA effect (48). *H. pylori* also suppresses T cell proliferation and activation and induces selective T cell apoptosis, again partially through specific VacA effects on signaling (49, 73, S64–S66). It evades host adaptive responses by mimicry of the gastric epithelial fucosylated (Lewis) antigens (74, S67), and by antigenic variation of surface proteins including a critical pilus molecule, CagY (75). As this high-frequency antigenic variation occurs through mutation (usually slipped-strand mispairing) and intragenomic recombination between homologous sequences (19, 23, S9, S68), these genetic mechanisms are important contributors to immune evasion. Finally, *H. pylori* can also suppress less specific immune mechanisms such as phagocytosis (47, 76). The relative contributions of the different host manipulation and evasion strategies to *H. pylori* persistence are not established, possibly differing in individual hosts, but the existence of these varied mechanisms implies that immune surveillance of the gastric lumen is powerful, and that bacterial survival requires its subversion.

Host responses to *H. pylori* and their role in disease

The immune response to H. pylori and its importance in pathogenesis. Despite the mechanisms *H. pylori* has evolved to avoid and downregulate host immune responses, substantial immune activation occurs following *H. pylori* infection. This is manifested by continuous epithelial cell cytokine signaling and gastric mucosal infiltration by neutrophils, macrophages, and lymphocytes, all of which are more pronounced in colonization with a *cag*⁺ strain (25, 65, 77, S69). There is a pronounced specific acquired immune

response, including generation of antibodies and effector T cells, and although this includes both a Th1 and a Th2 component, mucosal cytokine profiles imply Th1 predominance (72, S50). This is unusual for extracellular, toxin-producing bacteria, which usually are met by B cell activation and high-level antibody production (Th2 responses). However, studies in mice suggest that the predominant Th1 response is appropriate to control *H. pylori*: *Helicobacter* colonization density is lower in mice with predominant Th1 responses, whether genetically programmed or manipulated by experimental helminth infection (78, 79, S70, S71).

Despite its apparent propriety, the immune response, and in particular its Th1 component, is a major factor in *H. pylori*-associated pathogenesis (78, 80, 81, S70). Mice with a predominant Th1 response develop more gastric inflammation during *Helicobacter* colonization than those with a Th2 response (78, 79, 81, S70, S71). Experiments that use T cell transfer between mice show that these effects are dependent on Th1 cells (78). Gastric inflammation and atrophic changes are abrogated in the absence of the key Th1 cytokine IFN- γ (81, S70) and are induced by IFN- γ infusion, even without *Helicobacter* (S72). In humans, peptic ulceration is rare during immune suppression with cyclosporin A (S73) and pregnancy (S74), a Th2-predominant state. One hypothesis is that the relative sparsity of *H. pylori*-associated disease in Africa despite high *H. pylori* prevalence (the “African enigma”) may be due to predominant Th2 responses to *H. pylori* among black Africans. These responses may be induced by endemic helminth infection (79) or may reflect a genetic predisposition selected by malaria (82).

The importance of heterogeneity in immune responses among human populations and individuals is further demonstrated by the contribution of cytokine polymorphisms to disease risk. Polymorphisms that increase the IL-1 β response to *H. pylori* are associated with an increased risk of developing gastric atrophy, hypochlorhydria, and adenocarcinoma (83–85, S14, S75). Polymorphisms in TNF- α and IL-10 genes have a similar, but less pronounced, association (S14, S76). Thus the degree of activation of the immune response, which underlies *H. pylori*-associated pathology, is dependent on both *H. pylori* strain determinants and host genetic factors; the combined effect of these on disease outcome appears synergistic (S14), as predicted by the equilibrium model (Figure 1).

Effect of H. pylori-induced inflammation on acid homeostasis and its importance in upper-gastrointestinal diseases. *H. pylori*-induced proinflammatory cytokine expression and inflammation affect various cell types in the stomach that are important in acid homeostasis, including somatostatin-producing D cells, gastrin-producing G cells, and acid-producing parietal cells (86, 87, S77–S79). *H. pylori* gastritis causes a reduction in somatostatin levels (87, S77, S80) and, since somato-

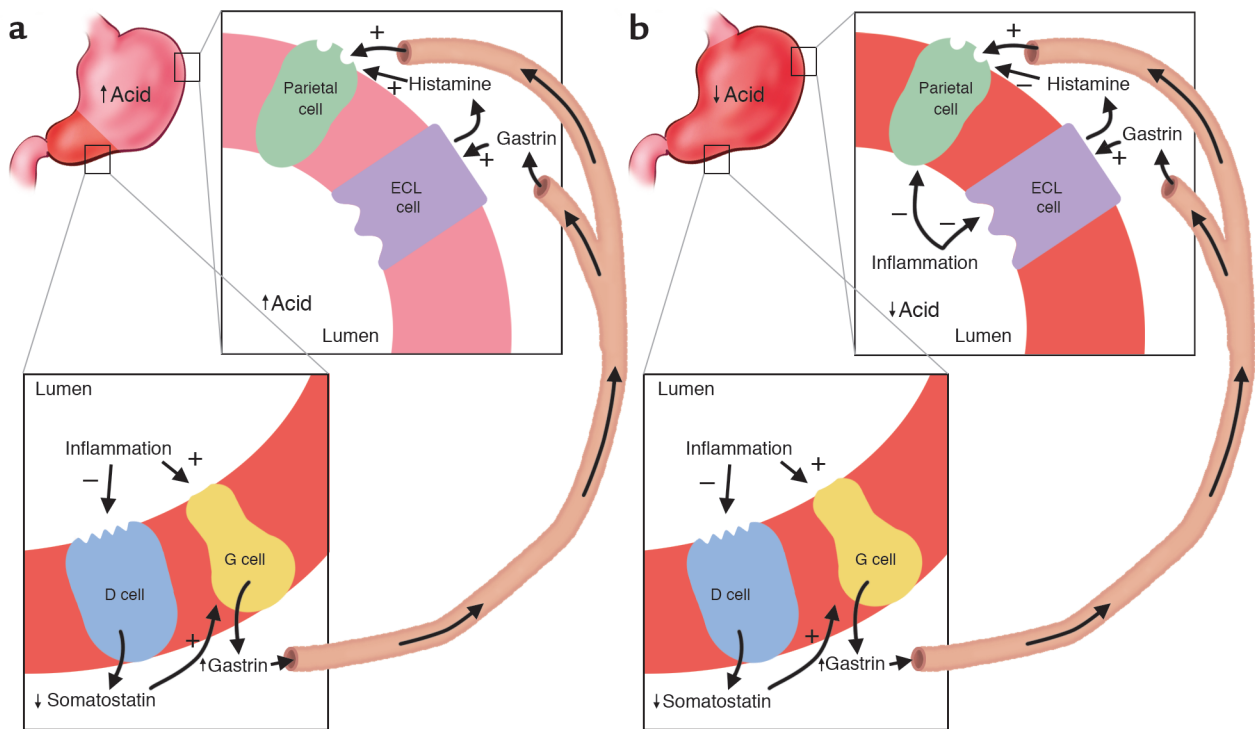


Figure 4

Relation of topography of inflammation to gastric physiology and clinical outcome. (a) *H. pylori*-induced antral-predominant inflammation. Antral inflammation results in hypergastrinemia, which stimulates a physiologically intact corpus to secrete acid, increasing risk for duodenal ulceration. (b) *H. pylori*-induced pan-gastritis. The inflammatory process suppresses corpus acid production, despite the gastrin stimulus from the antrum. Hypochlorhydria enhances risk of gastric ulcer and adenocarcinoma but conversely decreases risk for severe gastro-esophageal reflux disease and its sequelae. ECL, enterochromaffin-like.

statin negatively regulates gastrin, hypergastrinemia (88). Gastrin is a specific growth factor for *H. pylori* (89), so this potentially creates a positive-feedback loop. Gastrin expression may be enhanced by a direct stimulant effect of *H. pylori*-induced proinflammatory cytokines on G cells (S78). Removal of *H. pylori* reverses these effects (S81, S82) (Figure 4).

The effects of gastrin levels on acid homeostasis and disease depend crucially on the topographic distribution in the stomach of the *H. pylori*-induced inflammation (Figure 4). In antral-predominant gastritis, the enterochromaffin-like and parietal cells in the gastric corpus are relatively uninvolved; thus, high gastrin levels lead to greater acid secretion (90, S83, S84). Persistently increased gastrin levels also increase parietal cell mass, enhancing these effects (90, 91). The increased acid load delivered to the duodenum induces gastric metaplasia, a protective phenotypic change. *H. pylori* cannot colonize the normal duodenum but colonizes gastric metaplasia, with resultant inflammation and ulceration (92–94, S85). When the inflammation involves the corpus (pan-gastritis or corpus-predominant gastritis), *H. pylori*-induced inflammatory mediators suppress acid production both indirectly, by inhibiting enterochromaffin-like cell histamine production, and directly by inhibiting parietal cell function (86, S79). Reduced

acid secretion further augments gastrin levels, which, while ineffective in raising acid production from the inflamed gastric corpus, provide an ongoing proliferative stimulus to gastric epithelial cells. Continuing proliferation and inflammation affect epithelial cell cycle characteristics (95, 96, S86–S88) and lead to progressive loss of gastric glands. Such atrophic changes markedly increase risk of gastric ulceration and non-cardia gastric adenocarcinoma (4, 97, S89) but, because acid production is lowered, are protective against duodenal ulceration, and probably against acid-induced complications of gastroesophageal reflux (98, 99).

The topographic distribution of gastritis during chronic *H. pylori* colonization is at least partly host specific; for example, polymorphisms leading to high IL-1 β production are associated with pan-gastritis with its accompanying reduced acid production and gastric atrophy (84). However, environmental factors likely also play a crucial role; duodenal ulceration (and so presumably antral-predominant gastritis) is largely a 20th-century disease (100, S90) associated with socioeconomic development. Prior to 20th-century increases in duodenal ulcer incidence, the predominant gastritis pattern was probably that found commonly today in developing countries: pan-gastritis and progressive atrophy. As humans have coevolved

with *H. pylori* over at least thousands of years (8, 9) and our genes cannot have evolved appreciably over the last century, unknown environmental influences such as older age at acquisition, reduced number of colonizing strains, changed proportion of strains preadapted by passage through family members, reduction in other microorganisms colonizing the stomach, and improved nutritional status must be responsible for this change. In even more recent times, absence of *H. pylori* from late 20th- and 21st-century stomachs in developed countries, perhaps for the first time in our evolutionary history, may have had further effects on human acid homeostasis and health. As the predominant historical result of colonization was pan-gastritis and reduced acid production, absence of *H. pylori* would be expected to increase mean acid production in the general population, and we speculate that this has contributed to the observed rise in acid-associated complications of gastro-esophageal reflux (severe reflux esophagitis, Barrett esophagus, and esophageal adenocarcinoma) in the late 20th century (101). In support of this, patients with severe or complicated acid-esophageal diseases have a reduced *H. pylori* prevalence, particularly of *cag*⁺ strains (101–103, S91), and a low prevalence of gastric atrophy (98, 99). Consequently, the current iatrogenic *H. pylori* removal may have important costs as well as benefits.

The ubiquity of *H. pylori* in unacculturated human populations has led to speculation that colonization also may be beneficial to the pre-reproductive host (S92, S93). We speculate that over the long course of human evolution, adult stomachs were mainly atrophic, and antral-predominant gastritis and hyperchlorhydria were largely conditions of childhood; children

would be most likely to benefit from *H. pylori* colonization, by an enhanced acid barrier protecting against diarrheal pathogens (104). If so, there would be strong selection for maintenance of *H. pylori* in populations with poor sanitation; with improvement, such selection would be progressively lost.

Effects of H. pylori on leptin and ghrelin, hormones involved in appetite and satiety. Recently, gastric *H. pylori* colonization has been shown to affect expression of leptin and ghrelin, hormones that control appetite and satiety (5, 6, S94) (Figure 5). Leptin is secreted from adipose tissue and from the stomach (S95, S96); gastric leptin is produced by chief and parietal cells, and released in response to meals and associated hormonal stimuli (105, S96, S97). Leptin signals satiety to the hypothalamus, causing reduced food intake, increased energy expenditure, reduced gastrin and acid secretion, and increased gastric mucosal cell proliferation (106, S98, S99). Ghrelin, produced in oxyntic glands, is released during fasting, and suppressed by feeding and leptin (107, 108). In rats, ghrelin stimulates food intake, reduces energy expenditure, and increases acid secretion (107, S100).

Gastric leptin levels are higher in *H. pylori*-colonized than in noncolonized adults, and eradication leads to their reduction, although serum levels may not be affected (5, S94). Evidence conflicts as to whether serum ghrelin levels are higher in *H. pylori*-negative persons (109, S101), but they increase after *H. pylori* eradication (6). In an animal model, immunity to *Helicobacter* is associated with upregulation of adipocyte genes, including adipisin, resistin, and adiponectin (110). Inquiry in this field is at an early stage, but if early findings are confirmed, the implications may be important. Weight gain after *H. pylori* eradication is

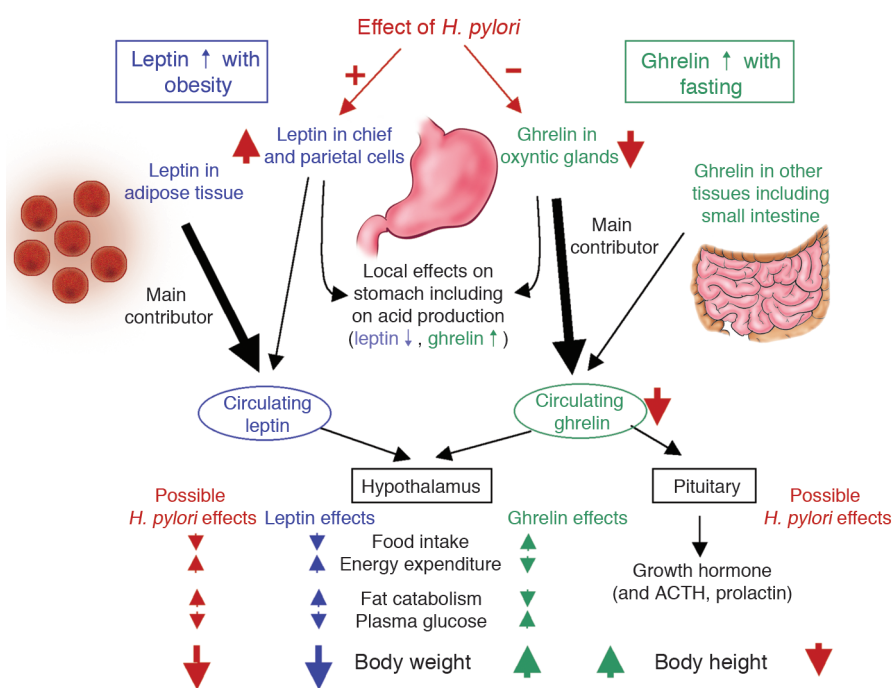


Figure 5

The described effects of *H. pylori* on leptin and ghrelin, and postulated subsequent effects on satiety, energy expenditure, weight, and height. Although leptin and ghrelin have other important paracrine, autocrine, and endocrine effects, here we concentrate on actions that affect body habitus. The observed effects of *H. pylori* on leptin and ghrelin are based on observational and interventional (*H. pylori* eradication) human studies. Other observational human studies support the portrayed effects of *H. pylori* on weight and height.

common (5), and these changes in hormonal levels may contribute (Figure 5). Similarly, obesity is increasing in developed countries, as *H. pylori* prevalence is falling. In developing countries, most children acquire *H. pylori* by age 5, and nearly all by age 10, whereas progressively fewer children in developed countries are becoming colonized (111, S102). Whether *H. pylori* genes represent microbial contributions to the complement of “thrifty” (calorie-conserving) genes of humans, and whether *H. pylori* disappearance plays a role in childhood (and adult) adiposity, remain to be determined.

Chronic effects of *H. pylori* on the gastric epithelium and carcinogenesis. Persistent microbial colonization resulting in inflammation and cell damage is an important cause of carcinogenesis. Examples include *H. pylori* and distal gastric adenocarcinoma, *Schistosoma haematobium* and bladder carcinoma, and hepatitis B virus and hepatoma (112, S103–S105). In evolutionary terms, such cancers are probably neutral; their expression is mostly modern, possibly because of increased human lifespan, and they may be regarded as a cost of chronic colonization, which for gastric cancer occurs in about 1–3% of *H. pylori*-colonized persons.

H. pylori-induced gastric carcinogenesis is more likely when the interaction between *H. pylori* and the host is more interactive; inflammation is more intense and the effects on epithelial cells are more damaging (S106). This may reflect colonization by more interactive *H. pylori* strains: *cag*⁺ strains induce more inflammation and cell cycle effects (4), and *vacA* s1/m1 strains cause more direct epithelial damage (27, 58, 59, S105). Host cytokine polymorphisms enhance the inflammatory response (83, 84, S14, S75). Damaging environmental factors, such as smoking and high-salt diets, further increase risk, whereas diets high in antioxidants are protective (113, S89, S107).

Although risk factors for gastric cancer now are well established, the mechanism of carcinogenesis is less clear. Carcinomas arise in stomachs with pan-gastritis; the more common intestinal type occurs following progressive atrophy (with loss of glands and hypochlorhydria), intestinal metaplasia, and dysplasia (S89, S108), whereas the diffuse type (S109) may arise de novo from *H. pylori*-colonized mucosa (112). *H. pylori* colonizes the atrophic stomach poorly, and intestinal metaplasia hardly at all, suggesting that the bacteria may create the environment for intestinal-type gastric carcinogenesis (atrophy and hypochlorhydria) rather than causing the cancer directly. In support of this concept, mutations in gastric carcinoma appear random, as expected from nonspecific DNA damage from environmental carcinogens (114, S110).

Disturbance of the epithelial cell proliferation/apoptosis balance is considered a risk factor for gastric atrophy and for neoplastic transformation. When cocultured with epithelial cell lines, *H. pylori* are antiproliferative and proapoptotic (115, S111), although *cag* signaling is essentially pro-proliferative

(through MAPK signaling and expression of the transcription factor AP-1) (116, 117) and pro- and anti-apoptotic (through NF- κ B signaling) (70, 118). Animal models and human studies suggest that the net effect of *H. pylori* colonization is pro-proliferative and proapoptotic (95, 96, 119, 120, S87, S88). Pro-proliferative signaling increases cell replication and the chance of mutation, whereas apoptosis may be protective by inducing death of DNA-damaged cells. However, the consequences of both effects on the epithelial stem cell compartment is likely to be pro-proliferative (to replace apoptotic cells), potentially predisposing to senescence and atrophy or increased mutation and diffuse-type malignant transformation. Stem cell proliferation also may potentially arise more directly from *H. pylori*-induced hypergastrinemia, since gastrin is pro-proliferative for gastrointestinal epithelia; in *H. pylori*-infected gerbils, epithelial proliferation correlates well with serum gastrin levels (96).

Ultimately, carcinogenesis requires DNA damage, which can be induced directly by *H. pylori* products or indirectly through oxygen free radicals released by neutrophils (118, 121, 122). Gastric ascorbic acid, which neutralizes free radicals, is reduced in *H. pylori*-positive stomachs (S112), and *H. pylori* can also directly interfere with the epithelial mismatch-repair system (122). In the atrophic stomach, *H. pylori* colonization is sparse, but atrophy is associated with continuing epithelial proliferation and an inflammatory cell infiltrate. Reactive oxygen species survive longer in the low-acid environment of the atrophic stomach, and ascorbic acid concentrations remain low (123); colonization by oral and intestinal bacteria, which themselves can release reactive oxygen and nitrogen species, may occur. By leading to gastric atrophy, *H. pylori* may be permitting its replacement by more genotoxic bacteria in the postreproductive-age gastric niche.

While carcinogenesis may be merely an evolutionarily irrelevant consequence of *H. pylori* colonization, affecting individuals largely in their postreproductive years, we advance an alternative theory (124). Carcinogenesis may be one mechanism by which *H. pylori* and other commensal bacteria contributed to the fitness of premodern human populations, by the removal of senescent (postreproductive) individuals from the population in a programmed (“safe”) manner (124). This would lead to a selective advantage for colonized populations, as groups dominated by senescent individuals likely have reduced survival during times of scarcity, or epidemic disease.

Conclusions

The human body is teeming with microbes, especially bacteria, but their role in human physiology has been little explored (124, 125). *H. pylori* is unique in that it is both the major inhabitant of an ecological niche and is disappearing from human populations as a consequence of modernization. As such, the effects of *H. pylori* on physiology and pathophysiology

gy can be measured and are a paradigm for our persistent indigenous biota, with both local and distant physiological effects.

Other microbes also may be disappearing from the human “microbiome” (125). We cannot yet ascertain this phenomenon because of the complexity of the indigenous flora, but parallels likely are present. Could extinction of *H. pylori* and other coevolved microbes have affected our physiological signaling? If so, could this in part be responsible for diseases that have increased in modern times, such as gastroesophageal reflux, obesity, diabetes, asthma, and several malignancies?

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Note: Due to space constraints, a number of important references could not be included in this reference list. Interested readers can find a supplementary reference list at <http://www.jci.org/cgi/content/full/113/3/321/DC1>.

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