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# *Helicobacter pylori*: Bacterial Strategy for Incipient Stage and Persistent Colonization in Human Gastric Niches

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*Helicobacter pylori* (*H. pylori*) undergoes decades long colonization of the gastric mucosa of half the population in the world to produce acute and chronic gastritis at the beginning of infection, progressing to more severe disorders, including peptic ulcer disease and gastric cancer. Prolonged carriage of *H. pylori* is the most crucial factor for the pathogenesis of gastric maladies. Bacterial persistence in the gastric mucosa depends on bacterial factors as well as host factors. Herein, the host and bacterial components responsible for the incipient stages of *H. pylori* infection are reviewed and discussed. Bacterial adhesion and adaptation is presented to explain the persistence of *H. pylori* colonization in the gastric mucosa, in which bacterial evasion of host defense systems and genomic diversity are included.

**Key Words:** *Helicobacter pylori*, gastric infection, persistent colonization, pathogenesis

## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a Gram-negative spiral bacteria which exclusively colonizes the gastric mucosa of humans. The prevalence of infection ranges from 20% in developed countries to more than 90% in the developing world.[1] Most Korean people become carriers of *H. pylori* from early childhood,[2] and *H. pylori* infection is subsequently maintained for the entire lifetime if no chemotherapy is attempted for eradication of the bacteria. After infection, clinical manifestation can range from asymptomatic gastritis to serious disease, such as peptic ulcer disease and gastric cancer. Recurrent peptic ulcers may occur in about 30–40% of the infected population.[1] Approximately 1–2% of infected persons progress to the worst malady, gastric adenocarcinoma.[1] Humans have probably been infected with this bacterium for at least fifty thousand years.[3] Infection by *H. pylori* is mainly acquired during infancy via fecal–oral and oral–oral pathways in the family. It has been classified as a group I carcinogen by the International Agency for Research on Cancer since 1994.

*H. pylori* produces acute and chronic gastritis at the beginning of infection, and inflammatory responses persist in the gastric mucosa. The characteristics most strikingly mentioned in *H. pylori* infections are the infection of the stomach of humans and persistent colonization in the gastric mucosa after infection. Prolonged carriage

of *H. pylori* causes sustained inflammatory response in the gastric mucosa, resulting in the elicitation of many gastric maladies. How *H. pylori* infects and persistently colonizes the gastric mucosa will be highlighted in this review.

## HOSTILITY OF GASTRIC MUCOSA

The gastric lumen and mucosa have been known to carry a variety of biochemical factors which are hostile to microorganisms. The gastric lumen carries gastric juice of pH 1–2 during fasting periods, which prevents bacterial growth. Gastric acid is secreted by parietal cells under regulation accomplished by the coordination of neural, hormonal, and paracrine pathways.[4] After ingestion, the pH of the gastric contents increases to a value of around 5, which lowers to around 3 after 60 min, and further decreases to 2 or lower after 2 h.[5] *H. pylori* can grow in liquid media around pH 5.1, meaning the bacteria can sufficiently endure the acidic conditions of gastric ingesta to infect the stomach when ingested per-orally with food stuffs.

## HISTOLOGY OF GASTRIC MUCOSA

The gastric mucosa consists of a layer of secreted mucus and a single layer of columnar epithelial cells. A mucus layer completely covers the whole epithelial surface of the gastric mucosa. Mucosal epithelia form on the surface and the glands of the stomach. The stomach can be divided histologically into the cardia, fundus-corpus, and antrum and functionally into the oxyntic gland area and pyloric gland area. The cardia is a transitional region between the squamous mucosa of the esophagus and the oxyntic mucosa of the stomach. The fundus and corpus, occupying about 80% of the gastric mucosa, are localized by acid secreting parietal cells, mucus neck cells (MNCs), and pepsinogen-secreting zymogenic (chief) cells. The antrum is characteristically observed by gastrin-secreting G cells and gland cells.[6] The gland unit occurs in tubular forms which are comprised of a foveolus (a gastric pit), an isthmus, a neck, and a base.

## MUCIN

Mucus is constituted by mucin glycoproteins which are highly *O*-glycosylated proteins endowing strong viscosity. The central mucin domain has tandem repeats rich in proline, threonine, and serine, which harbor *O*-linked *N*-acetyl galactosamine as well as *N*-linked sulfate-bearing glycans. Mucin appears in fibers typically 10–40 MDa in size and 3–10 nm in diameter.[7] Each mucin monomer is 0.2–0.6 μm in length, and these monomers are linked together end to end by disulfide bonds.[8] The composition of *O*-glycans is determined by a set of host-specific glycosyltransferases. Glycan coverage of mucins with 25–30 carbohydrate chains per 100 amino acid residues occupies up to 80% of the dry weight of mucus.[7] Most mucin glycoproteins have a high sialic acid and sulfate content, which leads to a strongly negative surface that increases the rigidity of the polymer via charge repulsion.[7] Mucins are grouped structurally into membrane-associated and secreted mucins. Membrane mucin is cleaved into two subunits, *N*-glycosylated in endoplasmic reticulum (ER) and *O*-glycosylated in Golgi, and inserted into the membrane. Secreted mucin dimerized in ER and *O*-glycosylated in the Golgi is then oligomerized to form either linear polymers following N-terminal dimerization or a complex molecular networking followed by N-terminal trimerization.[7] Based on the form of oligomerization, secreted mucins can be classified into gel-forming and non-gel-forming mucins. The secreted mucus forms two layers: a thinner inner layer that is sterile and difficult to dislodge, and an outer layer that is not sterile and is more easily removed.[7] The gastric mucins are the membrane-associated MUC1 and the secreted gel-forming MUC5AC and MUC6.[9] Surface mucus cells mainly produce MUC5AC, which is the major structural constituent of both the inner and outer mucus sublayers. MUC6 is secreted by MNCs. The secreted mucus then adheres to the epithelial cells by both adhesive and entangling interactions with the cell-surface mucins. It has been demonstrated *in vitro* that membrane-associated MUC1 protects the epithelium against adhesion of *H. pylori*.[10] MUC1-deficient mice displayed higher colonization levels of *H. pylori* and more severe gastritis compared with wild-type mice.[11] The 200–500 nm long extracellular domain of MUC1 is capable of physically distancing the bacteria from the gastric epithelial surface, resulting in steric inhibition of adhesion to other potential cell surface ligands.[10] MUC6 was reported to inhibit the synthesis of *H. pylori* cell wall components, indicating a restriction of *H. pylori* growth within the gastric mucus.[12]

## PHYSIOLOGY OF MUCUS LAYER

A number of properties have been listed to explain the role

played by the gastric mucus layer in the protection of the epithelial cells and underlying host tissues from the luminal contents of the stomach, such as acidic juice, pepsin, and toxin intakes, as well as from infection by pathogens. The gastric mucus layer has a depth of 190–300 μm, which completely covers the surface of all gastric epithelia, separating the epithelia from the gastric lumen at all times without any gaps under physiological conditions.[13] Mucus is produced and moves outward at a velocity of 1–100 μm/s, secreted into the gastrointestinal (GI) tract at a volume of 10 L per day.[14] Continuous secretion and clearance by peristaltic forces lead to quick turnover times, on the order of 4–6 h.[15] Mucus has the viscoelastic ability to maintain an unstirred layer adjacent to epithelial surfaces, despite the vigorous shearing actions. The bulk viscosity of healthy human mucus is typically 1000–10000 times higher than the viscosity of water at a low shear rate.[8] Viscoelasticity is endowed by high content of sialic acid and sulfate in the mucin and is dependent on the concentration of mucin.[8] Gastric mucus has sufficient viscoelasticity to block the motility of many types of bacteria. It was reported that neutrophils can migrate through mucus with a dry weight of 1.5–2.5%, but not with a dry weight of 6.5%.[16] Neutrophils have not been observed in the gastric mucus layer, despite infection by *H. pylori*, indicating that the viscoelasticity of the gastric mucus layer is high enough to block the migration of neutrophils. Whereas soluble globular protein can diffuse freely through mucus, secreted antibodies are slowed by about 2-fold. Mucin fibers are long strings consisting of a flexible array containing periodic hydrophilic regions with a negative charge (carboxyl or sulfate groups), relatively hydrophobic regions, and highly hydrophilic regions.

Exposing gastric mucin to pH <4 greatly increases the presence of hydrophobic surfaces, causing aggregation of the mucins. In fact, mucus was observed to significantly decrease the diffusion of a number of both small and large molecules, including tertiary amines, bovine serum albumin, lysozyme, and others.[8] In addition, hydrophobic interactions also represent an important mechanism by which mucus limits the transport of larger entities such as bacteria.[8] Mucus itself has been known to be insufficient for protecting the surface of gastric epithelia from the corrosive acidity of gastric juice. However, mucus on the surface of the mucosa provides an unstirred layer in which bicarbonate secreted by the surface epithelium makes a pH gradient which neutralizes the hydrogen ions diffusing towards the epithelium from the lumen. The hydrogen ion concentration on the epithelial side of the mucus layer is $1.1\times10^{-4}$ mM (pH 6.96) when the luminal hydrogen ion concentration is 5.6 mM (pH 2.25), meaning that human gastric mucus is capable of maintaining a concentration gradient of hydrogen ions through the mucus-bicarbonate barrier.[17] Although the human stomach can secrete 40 mM or more of acid, which overwhelms the gradient of the mucus layer, this is rarely maintained within the gastric lumen for any length of time under physiological conditions.[17]

Taken together, the gastric mucus layer is a complete barrier for blocking the diffusion of solutes and particles, as well as the infection of pathogens other than *H. pylori* from the luminal ingesta. However, the mucus also plays the role of completely protecting the *H. pylori* colonized in the mucus layer, not only from acidic juice and antimicrobial agents diffused from the gastric lumen but also from host defense factors like humoral and cellular immunity. Therefore, the gastric mucus layer provides sufficient niches for prolonged survival and growth of *H. pylori*.

## HOW CAN *H. PYLORI* INFECT AND COLONIZE THE GASTRIC MUCUS LAYER?

It has been well documented that *H. pylori* infection occurs in early childhood, but rarely in adults. Gastric hostility in adults sufficiently inhibits *H. pylori* infection. Most epidemiological studies have shown that *H. pylori* infection rates continue to rise with age.[2] The rising prevalence with increasing age may be explained by a cohort effect.[2,18] The prevalence of *H. pylori* infection is different with regions and countries, which may be caused by the different rates of *H. pylori* acquisition at early childhood.[19] Why *H. pylori* infection occurs in early childhood has not yet been sufficiently explained. The ontogenic development of the stomach in early childhood life is thought to be associated with the alleviation of gastric hostility, including achlorhydria.[20]

## NEUTRALIZING FACTOR OF *H. PYLORI* AGAINST GASTRIC ACIDITY

*H. pylori* must overcome the acidic lumen of the stomach until penetration of the gastric mucus layer has been achieved after oral ingestion, after which it must also survive in the pH gradient of the gastric mucus layer after penetration. It

has been observed that *H. pylori* has enhanced the synthesis of urease, formamidase, and arginase when meeting an acidic environment. These enzymes are associated with the production of ammonium ions. *H. pylori* produces a large amount of potential urease enzyme which consists of 6–10% of bacterial proteins and has a *Km* value for urea of 0.8 mM, meaning that it is able to hydrolyze the limited amounts of urea available in the stomach to generate ammonia and $CO_2$, leading to an increase in the pH nearby the bacterium. Injection of *H. pylori* whole lysate is lethal to mice because of the ammonium toxicity elicited by urease.[21] The 8.6 kb of *H. pylori* urease genes are organized into two structural genes, *ureA* and *ureB*, and five accessory genes, *ureI*, *ureE*, *ureF*, *ureG*, and *ureH*. Urease is a nickel-binding hexamer of UreA and UreB. Accessory subunits are associated with urea absorption and nickel incorporation into the UreB subunit. Coexpression of structural genes and accessory genes produce enzymatic metalloproteins, which otherwise exist as inactive apoproteins. UreF, UreG, and UreH are all required for the synthesis of an active urease.[22] *H. pylori* urease is an unsecreted cytoplasmic protein, which therefore plays a role in the regulation of internal proton concentration by increasing the periplasmic pH and membrane potential.[23] UreI is a proton-gated urea channel with acidic activation, which regulates cytoplasmic urease activity by the intracellular import of urea.[24]

In addition to urease, aliphatic amidase and formamidase produce ammonia and the corresponding organic acid by hydrolyzing short-chain amino acids, while arginase hydrolyzes L-arginine to L-ornithine and urea.[25] These ammonia-producing enzymes including urease are regulated in response to low pH by the ArsSR two-component system which consists of the histidine kinase ArsS responsible for sensing low pH, an OmpR-like response regulator, ArsR.[25]

## HOW DOES *H. PYLORI* PENETRATE THE GASTRIC MUCUS LAYER?

*H. pylori*-contaminated ingesta in the gastric lumen needs to penetrate and swim into the viscoelastic mucus layer of the gastric mucosa in order to establish persistent infection. The extent of mucus viscoelasticity is dependent on pH,[26] while the glycoprotein content is primarily responsible for its viscoelasticity. Gastric mucus undergoes a reversible pH-dependent transition between a soft gel and a viscous polymer. The glycoprotein macromolecules of mucin change to the solution phase at a neutral pH and to the gel phase at a low pH (<4).[26] Mucus is gelled slightly stronger at pH 2 than at pH 4. However, *H. pylori* achieves penetration by altering the rheological properties of the mucus layer. As the pH of the gastric lumen becomes more acidic, the mucus layer becomes more viscoelastic and aggregated. *H. pylori* urease produces $NH_3$ in the mucus-containing urea. It is possible for the $NH_3$ to diffuse to ≈20 μm of radial separation in less than ≈70 ms, with an estimated diffusion constant of $D \approx 10^{-9}$ $m^2/s$, causing the widespread pH elevation to liquefy the mucus in the vicinity of each bacterium.[27]

### Helical shape

*H. pylori* is a spiral bacterium, and this helical shape is thought to enhance motility through the viscoelastic mucus layer by corkscrew propulsion. Helical bacteria such as *H. pylori* and *Campylobacter* can swim faster than straight-rod *E. coli* in liquid cultures presumably due to their helical body shape.[28] Helical shape is established by the sum of cell elongation, curvature, and twist.[29] The curvature structure is formed by peptidoglycan cross-linking relaxation associated with the gene products of *ccd1*, *ccd2*, *ccmA*, *ccd3*, *ccd4*, *ccd5*, and *ccd6*. The Ccd proteins play the role of DD-endopeptidases or carboxypeptidases. Csd1, Csd2, and CcmA may dissolve the tetrapentapeptide crosslinking of peptidoglycans to support helical twist and cell curvature. Csd3 induces changes in the level of cross-linked muropeptide content to maintain a helical form.[29] Csd4 has DL-carboxypeptidase activity on tripeptide monomers, cleaving the terminal mDap residue to produce dipeptide monomers. Ccd6 has LD-carboxypeptidase activity and seems to act in concert with Csd4 to produce dipeptides.[30] Csd4 and Csd6 proteins have opposite effects on the conversion from tripeptides to dipeptides, related to the induction of cell curvature.[30] Csd5 might provide an activating signal for cell shape modulation, perhaps through recruitment of enzymes involved in peptidoglycan synthesis, such as Csd4 and Csd6. All deletion mutants for these genes showed loss of helical shape and demonstrated a decrease in the infectivity to murine gastric mucosa.[29,30] In addition, *H. pylori* contains a complex cytoskeleton which affects cell morphology as well as motility.[31] It possesses four coiled-coil rich proteins, Ccrp58, Ccrp89, Ccrp1142, and Ccrp1143, which extend filamentous structures and control cell shape and motility.[31] The deletion of these Ccrp genes caused a loss of the helical shape and decrease of motility.[31] Ccrp filaments may be incorporated with proteins which synthesize the cell wall,

and Csd proteins form the helical shape during bacterial growth.

**Flagella**

The mobility of *H. pylori* is conferred by flagella. Motile flagella are critical for the persistence of *H. pylori* in the gastric mucus layer, meaning they endow the locomotion ability of the organism in the mucus layer.[32] *H. pylori* carries a polar tuff of three to five sheathed flagella, 4.3–9.5 μm long and 23.5–40 nm thick. A characteristic terminal bulb is observed, which may be an extension of a flagellar sheath. Under electron microscopic observation, the flagella appear to be 1.35-nm thick filaments surrounded with sheaths, showing the typical bilayer structure of a membrane containing lipopolysaccharide (LPS) and several proteins.[33] *H. pylori* flagella are synthesized by the coordination of over 40 genes dissipated in the whole genome. Bacterial flagella consist of a basal body, hook, and flagellar filament. The basal body is composed of the LP ring (FlgH and FlgI), the MS-C ring (FliF, FliG, FliM, and FliN), and the rod (FliE, FlgB, FlgC, and FlgG).[32] The LP ring is assembled at the level of the outer membrane and peptidoglycan. The MS ring (M, membrane; S, supramembrane) is built up by the self-assembly of FliF above and within the cytoplasmic membrane.[34] The cytoplasmic face of the MS ring is mounted with the drum-shaped C-ring. The rod is a shaft which connects the LP ring and the MS-C ring. The center of the cytoplasmic face of the MS-C ring is located with the flagellar protein export apparatus. The flagellar filament is made of two flagellins: the major, FlaA, and the minor, FlaB.[34] FlaA builds up an extracellular region of the flagella. *H. pylori* flagellins were identified to be modified post-translationally by glycosylation. The FlaA protein is modified with a total of seven *O*-linked pseudaminic acid (Pse5Ac7Ac) residues, while FlaB is modified with ten *O*-linked Pse5Ac7Ac residues. Pseudaminic acid is a nine-carbon sugar absent from humans and is biosynthesized by several genes. The deletion of any of these genes results in non-motile *H. pylori* that lack functional flagella, and subsequently, there is a loss of mouse infectivity, demonstrating that the pseudaminylation of flagella subunits is absolutely essential for *H. pylori* to synthesize functional flagella to colonize the host's stomach.[35]

Bacteria move by a rotating movement of the rotary motor at the base of the filament in both clockwise (CW) and counterclockwise (CCW) directions.[34] The basal body imbedded in the cytoplasmic envelope works as a reversible rotary motor, while the hook and filaments extended extracellularly act as a propeller. The basal body also includes the flagellar protein export apparatus, which consists of six integral membrane proteins (FlhA, FlhB, FliO, FliP, FliQ, and FliR) and three soluble proteins (FliH, FliI, and FliJ). The export of LP ring is carried out by a Sec-dependent pathway. And the extracellular components of the flagellum may be transported by a central channel of the on-going structure by a type III secretion mechanism.[32] FliI is associated with provision of the energy required for the export process.[32]

The rotary motor is powered by proton or sodium ion motive force across the cytoplasmic membrane. The motor is made of a rotor (MS-C ring), a drive shaft (rod), a bushing (LP ring), and about a dozen stators. The rotor (rotating part) is surrounded by a circular array of the stator complexes (nonrotating part) in the membrane. The stator complexes are formed by the MotA and MotB proteins, in which MotA is inserted into the cytoplasmic membrane and MotB contacts with the peptidoglycan. The C-ring is assembled with FliG, FliM, and FliN to be termed the switching complex, producing both rotational movement and CW-CCW switching.[36] FliG is the rotor protein most directly involved in the generation of torque. The C-terminal domain of FliG forms the rotor portion for the site of energy transduction in the flagellar motor and interacts with the stator protein MotA.[37] *H. pylori* demonstrates positive chemotaxis towards urea, bicarbonate, and body fluid. *H. pylori* senses chemotactic attractants through the membrane-bound methyl-accepting chemotaxis proteins (MCPs) TlpA, TlpB, TlpC, and HP0599, which transduce the signals to CheA. CheA is stimulated by the MCPs to become autophosphorylated via CheW, and transfers a phosphoryl group from a histidine residue to an aspartate of CheY. The phosphorylated CheY then interacts with FliM to change the direction of motor rotation with CW-CCW switching.[38]

After ingestion, the gastric mixture becomes weakly acidic, to which *H. pylori* is tolerant. *H. pylori* attaching to the gastric mucus layer uses urease to hydrolyze urea in the mucus layer for the production of $NH_3$. $NH_3$ rapidly diffuse to the mucus layer in the vicinity of *H. pylori* to change the pH, which modifies the rheologic properties of the mucin network. Thereafter, *H. pylori* achieves penetration into the gastric mucus layer by coupling the corkscrew propulsion originating from the helical shape with flagellar motility, which is obtainable due to the liquefied mucin in the vicinity of *H. pylori*.

## ADHESION FACTORS OF *H. PYLORI*

When biopsy specimens of patients with chronic gastritis were examined after staining, *H. pylori* were observed within and beneath the gastric mucus layer, 20% of which were attached to the epithelial surface of the gastric mucosa.[39] As mentioned above, gastric mucus moves rapidly outward and is secreted into the GI tract; thus, *H. pylori* must overcome the shedding flow of mucus by the locomotive force of the flagella and the use of an adherent mechanism attaching to the epithelium of the gastric mucosa. The adherence of *H. pylori* to the gastric mucosa plays a critical role in initial colonization and long-term persistence, as well as in the pathogenesis of gastric disorders in the gastric mucosa of humans.

Ultrastructural observations demonstrated *H. pylori* to be located at the tip of mucosal microvilli, bound to the pedestal-like structure of epithelial cells, showing rearrangement of the actin cytoskeleton or associating strongly with the epithelial cell membrane via filamentous material.[40] Several candidates of cellular receptors for *H. pylori* binding have been suggested, including sialic acid-containing molecules, laminin, phosphatidylethanolamine, and the Lewis B antigen. *H. pylori* ligands for these cellular receptors were purified by several research groups. A fibrillar N-acetylneuraminyllactose (NL)-binding hemagglutinin (NLBH) corresponding to HpaA was first identified to be responsible for the adherence of *H. pylori* to mammalian cells in tissue culture. HpaA is localized on the surface of *H. pylori*, including on the flagellar sheath. HpaA is known to be essential for bacterial colonization of the gastric mucosa.[41] In addition, *H. pylori* was found to specifically recognize gangliotetraosylceramide, gangliotriaosylceramide, and phosphatidylethanolamine *in vitro* via a 63-kDa exoenzyme S-like adhesin, which is responsible for the lipid-binding specificity. A 19.6-kDa iron-binding protein showed apparent binding activity for erythrocytes, human buccal epithelial cells, and laminin, but the binding is likely nonspecific.[42] Although these proteins of *H. pylori* bind to mammalian cells *in vitro*, their roles as adhesins to the gastric epithelial surface have not yet been demonstrated *in vivo*.

### LPS

The outer envelope components of *H. pylori*, LPS and outer membrane proteins (OMPs) are closely associated with its adherence to host cells. The most outer envelope of *H. pylori* contains LPS, as do those of other Gram-negative bacteria.[43] Generally, LPS is the component of Gram-negative bacteria that contributes to immune modulation and biological toxicity associated with the pathogenesis of bacterial infections. In enterobacteria, LPS consists of lipid A, core oligosaccharide, and O-chains of repeating units. The typical lipid A backbone of enterobacteria is a β, 1'-6-linked disaccharide of glucosamine which is bisphosphorylated and multiply acylated with (*R*)-3-hydroxymyristate at the 2-, 3-, 2'-, and 3'-positions. Core oligosaccharide is a branched polysaccharide of 9–12 sugars, including the unusual sugar 2-keto-3-dexoxy-octanoate (KDO), that covalently binds to the glucosamine ring of lipid A. The O-side chain is a long, branched, or linear polysaccharide consisting of 50–100 repeating units, which bind the core oligosaccharide.[43] The LPS of *H. pylori* is synthesized by the incorporation of at least 27 genes, which are scattered throughout the whole genome and are unlike other bacteria, with the exception of *rfbM*, *rfbD*, and *wbcJ* orthologs encoding proteins involved in GDP-fucose synthesis.[44] Orthologs to all enzymes required for lipid A and core oligosaccharide synthesis have been identified in the *H. pylori* genome. The lipid A of *H. pylori* lacks a phosphate group at the 4'-hydroxyl position on the distal nonreducing glucosamine of the disaccharide, is substituted at the C-1 position on the proximal glucosamine with a phosphoethanolamine residue, and is tri-acylated or tetra-acylated with either (*R*)-3-hydroxystearate (C18) or (*R*)-3-hydroxypalmitate (C16).[45] During the synthesis of *H. pylori* LPS, *H. pylori* WaaA transfers two KDO sugars to the distal glucosamine of the tetra-acylated lipid A precursor lipid $IV_A$, followed by the addition of the acyloxyacyl-linked fatty acyl chains, resulting in a minor lipid A.[46] After constitutive biosynthesis, Kdo2-lipid A is modified by the removal of the 1-phosphate group from lipid A by lipid A-1 phosphatase (HP0021) on the periplasmic side of the inner membrane, after which LPS is transported across the inner membrane. The removal of the outer KDO sugar or the addition of a phosphoethanolamine residue to the 1 position is carried out by phosphoethanolamine transferase (HP0022) in the periplasmic space.[46,47] Conclusively, *H. pylori* LPS shows significantly lower immunologic activity and endotoxicity compared with enterobacteria due to underphosphorylation, underacylation, and substitution with long chain fatty acids in its lipid A. In addition, modification at the 1 position of *H. pylori* lipid A confers resistance to cationic antimicrobial peptides like polymyxin B. A core region with an unusual branching patterns and O-chains of relatively constant chain length are found in *H. pylori* LPS.[48] One of the characteristic features of *H. pylori* LPS is the abun-

dance of D-*glycero*-D-*manno*-heptose (DD-heptose) residues in its core region, unlike other Gram-negative bacteria, which mainly contain L-*glycero*-D-*manno*-heptopyranose (LD-heptose). *H. pylori* HP0479 was identified to encode a D-*glycero*-D-*manno*-heptosyltransferase gene which adds a D-*glycero*-D-*manno*-heptose residue (DDHepII) to a distal DD-heptose of the core oligosaccharide backbone of *H. pylori* LPS.[49]

The *H. pylori* O-chains are assembled in the cytoplasm onto a polyisoprenoid membrane anchor. WecA, which plays the role of the UDP-GlcNAc:undecaprenyl-phosphate GlcNAc-1-phosphate transferase, assembles the glycan of Gal and GlcNAc residues, generating the linear O-chain backbone. Fucosyltransferases then decorate fucose residues to specific locations of the O-chain backbone, forming Lewis antigens.[50] The flippase Wzk translocates the O polysaccharide to the periplasm, where the O antigen ligase WaaL connects it to the lipid A-core to assembe the LPS molecule. The LPS molecule can then be transferred to the outer membrane to appear in the outer side of the bacterium.[50]

The most striking observation about *H. pylori* LPS was the identification of Lewis X in its O-chain, followed by the discovery of the mimicry of type 1 and type 2 blood group antigenic determinants on the core oligosaccharide. Some strains express sialyl-Lewis X in the O-chain. Additionally, novel sugars of L-rhamnose, D-rhamnose, or 3-C-methyl-D-mannose, as well as heptoglycans composed of 2-linked and 3-linked D-*glycero*-a-D-*manno*-heptopyranose units, were found in the O-chain of some strains, revealing that not all *H. pylori* strains express Lewis antigen mimicry.[51]

The O-chains of *H. pylori* LPS are posttranslationally decorated with fucose residues to generate Lewis antigens. The precursor of type 2 Lewis antigens, LacNAc (*N*-acetyl-β-lactosamine, Gal β1-4GlcNAc), is synthesized by *rfaJ* homologues (HP0159) and HP0826, encoding β1,4-galactosyltransferase (β1,4GalT).[51] There are three fucosyltransferases in the genome: FutA, FutB, and FutC. Of these, FutA and FutB (α1,3/4-fucosyltransferases, α1,3/4-FucT) catalyze the transfer of GDP-fucose on nonreducing ends and internal sites of O-antigen polymers of various sizes to produce the Lewis X epitope, a substrate for FutC (α1,2-FucT) that yields difucosylated Lewis Y.[52] FutA and FutB, each of which contains a C-terminal heptad repeat region, are expressed under the regulation of on/off status via slipped-strand mispairing in the intragenic polyC tract regions of their genes.[53] The C-terminal heptad repeat region consists of a variable number of DD/NLRV/INY tandem repeats in which variation correlates to the size of the O-antigen polymers, which are decorated by fucose residues.[53] The expression of FucC is regulated by frame shifts in a polyC tract situated in the middle of the gene.[53] Type 1 precursor, Gal β1-3GlcNc, is decorated by FutA and FutB to produce Lewis A antigen and by FutC to produce H type 1 antigen. Lewis A is a substrate of FutC, which produces Lewis B.[53] Most *H. pylori* strains (>80%) have serologically been identified to express type 2 antigen Lewis X and/or Y, but a minor portion of the strains (<10%) were shown to carry type 1 antigen Lewis A and B.[54] East Asian strains express both type 1 and type 2 antigens far more frequently compared to others.[55] The Lewis antigens of *H. pylori* LPS show extensive diversity over the infection periods and in the colonizing regions of the host stomach *in vivo*, as well as over the duration of culture and according to culture conditions *in vitro*.[56] *H. pylori* was observed to adapt to the host by phase variation of its LPS, in which the bacterial Lewis antigen evolves to resemble the host's gastric Lewis phenotype, demonstrating that the bacterial genetic locus is subjected to host-driven selective pressure.[56] Such phase variation could be explained by regulation of the on/off status of fucosyltransferase genes.

*H. pylori* LPS plays several delicate and complicated pathogenic roles to allow persistent infections of the gastric mucosa, of which the foremost function is adherence to the gastric mucosa. Lewis antigen mimicry was suggested to be implicated initially in immune evasion and gastric adaptation by the bacterium. However, long-term *H. pylori* infection can induce the production of cross-reacting autoreactive anti-Lewis antibodies, which is reported to react to the β-chain of the gastric proton pump ($H^+$,$K^+$-ATPase) contained within parietal cell canaliculi, contributing in part to the development of gastric atrophy.[57] The Lewis X antigen in the O-chain is involved in the adhesion of *H. pylori* to the human antral gastric mucosa through the gastric receptor of the galactoside binding lectin, galectin-3.[58] In addition, H type 1 antigens are suggested to attach to a membrane mucin MUC1, through carbohydrate-carbohydrate interaction.[59] Lewis antigen mimicry in the O-chain contributes directly to the development of bacterial pathogenesis, but not to bacterial colonization. To date, no investigations have been done on the circumstances under which Lewis antigen mimicry in the O-chain of LPS could mediate the adherence of bacteria to the gastric mucosa.

**Outer membrane protein (OMP) adhesins**

In addition to LPS, the *H. pylori* outer membrane mediates

direct contact with the surrounding environment of the host, including the mucus and the epithelial surface of the gastric mucosa. Therefore, *H. pylori* OMPs have been postulated to be crucial for adaptation to and persistent colonization of the host. Of the 1600 ORFs in the *H. pylori* genome, approximately 4% have been annotated as OMPs, representing a significantly larger amount than in any other bacterial genome.[44] Of the ORFs, 64 were predicted to be OMPs, classified into 5 paralogous families. Of these, 33 belong to the Hop (Helicobacter outer membrane porins) and Hor (Hop-related proteins) groups.[60] The HOP subfamily includes several known *H. pylori* adhesins including BabA (HopS), SabA (HopP), AlpA (HopC), AlpB (HopB), HopZ, and OipA (HopH), which promote and enhance bacterial interaction with the epithelial surface of the gastric mucosa. These genes share high similarity or identical sequences at the amino and carboxy termini which could elevate the possibility of intragenomic or intergenomic recombination in the *H. pylori* population. The genes also contain dinucleotide (CT) repeats in their signal sequences, suggesting that their expression could be regulated by slipped-strand repair.

BabA, the *H. pylori* blood group antigen-binding adhesion, is the best-characterized of the *H. pylori* adhesins. It binds Lewis B antigen, a fucosylated blood-group antigen present on gastric epithelial cells. Human colostrum secretory immunoglobulin A was observed to inhibit the binding of *H. pylori* to gastric surface mucus cells, which is caused by digestion with α-L-fucosidase, implying that fucose-containing factors are involved in this inhibition rather than immunoglobulin.[61] The fucose-sensitive receptor for *H. pylori* was identified as Lewis B, a fucosylated carbohydrate structure that is the predominant blood group-related antigen expressed on gastric surface mucus cells in the stomach epithelium of individuals with positive secretor status.[62] BabA adhesin is located on the outer membrane at frequency of ca. 500 per cell with *Ka* value of $\approx 1\times10^{10}$ $M^{-1}$.[62] There are three BabA genes according to the strain (*babA1*, *babA2*, and *babB*), which differ by a repeating motif in the signal peptide sequence or translation start point. The signal peptide starts at position -20 in BabA and position -18 in BabB. The *babA2* gene responsible for the production of the functional BabA adhesin is identical to the *babA1* gene with the exception of a 10-bp insert with a repeat motif in the signal peptide sequence resulting in the creation of a translational initiation codon. Most isolates contain *babA* zero to two copies in the three possible loci of the chromosome.[63] Chimeric *babB/babA* alleles have been detected among clinical isolates, and frame shift mutation and polymorphisms are present throughout the *babA* alleles.[63] European and American strains (generalist strains) bind to blood group A, B, and O type 1 determinants, which are the most prevalent in the population. In contrast, native South American strains (specialist strains) bind only to the blood group O type 1 determinants (Lewis B and H type 1) of the local prevalent blood type.[64] In addition, during infection of rhesus macaques, *babA* is replaced by *babB* or not expressed at all because of alteration in the dinucleotide CT repeats in the 5' coding region, revealing that *babA* is regulated to facilitate adherence to the gastric epithelium and to promote chronic infection through both antigenic variation and phase variation.[65] These observations indicate that BabA adhesin progressively evolves in the direction for bacterial adaption to their individual hosts in order to sustain the extraordinary chronicity of gastric infection.[66] However, many strains do not contain *babA* bound to Lewis B antigen as well as Lewis B binding activity. Consequently, these strains do not reflect the severity of mucosal damage or the clinical outcome, implying that the expression of BabA adhesin is not critical for persistent colonization with *H. pylori* in the gastric mucosa of humans.

AlpAB, adherence-associated lipoprotein A and B, was found to be involved in adherence to the gastric cell line Kato III and is annotated as *omp20-omp21* in the 26695 genome and as *hopC-hopB* in strain G27.[67] AlpAB was identified as the abundant protein of the sarcosine-insoluble fraction.[68] AlpAB genes were identified by gene disruption using transposon mutagenesis and are encoded by two adjacent homologous genes organized in an operon with co-transcription. AlpAB has a porin-like b-barrel in the outer membrane, consisting of 14 transmembrane amphipathic β-strands. *H. pylori* strains lacking both *alpA* and *alpB* or containing only *alpB* have severely reduced laminin binding, demonstrating laminin to be a target of AlpB, while both are required for *H. pylori* laminin binding.[67] In Guinea pigs, *alpA* and *alpB* mutants could not be isolated from animals when infected with a mixture of the wild-type and mutant strains, and IgG antibody responses were lower in animals infected with either the *alpA* or the *alpB* mutants, suggesting that the absence of AlpA or AlpB presents a serious disadvantage for colonization of the stomach by *H. pylori*.[69]

SabA, a sialic acid-binding adhesion, was first observed as a receptor of gastric mucosa from an *H. pylori*-infected patient with gastritis, to which mutants of both *babA1A2* or only inactivated *babA2* could bind to.[70] Whereas the gastric

surface of patients infected with *H. pylori* can be bound by BabA mutants, that of healthy individuals without *H. pylori* infection cannot. A gastric surface target of SabA is sialyl-dimeric-Lewis X glycosphingolipid, which is induced in the gastric epithelium of humans and Rhesus monkeys by *H. pylori* infection. SabA was identified to be a 66 kDa protein encoded by JHP662 in strain J99 and HP0725 in strain 26695. The binding activity for sLewis X is spontaneously lost in 1% of the colonies expended from a single colony. Instability of sLewis X-binding may come from the regulation of the on/off frameshift modulated by poly T/CT tracts upstream, as well as from within the start of *sabA*. Although the on/off status of *sabA* was investigated to find no correlation with gastric disease, severe neutrophil infiltration and atrophy are associated with functional *sabA*.[71]

OipA, the outer inflammatory protein adhesin encoded by HP0638, is directly associated with the promotion of interleukin-8 (IL-8) production rather than binding ability to the gastric epithelial surface. OipA knockout mutant strains showed significant reduction in their binding abilities to AGS, and restoration of the adherences was shown in Kato-III by complementation of the *oipA* gene.[72] Although the presence of a functional *oipA* gene allows significant IL-8 secretion to be maintained above the control values even with disruption of *cagPAI*, it was supposed that OipA induces IL-8 secretion in conjunction with CagPAI. Live *H. pylori* induces IL-8 secretion though direct contact with gastric epithelial cells, in which regulation of transcription of the IL-8 gene is mediated by the interferon-stimulated responsive element (ISRE)-like element, activator protein (AP)-1, and NF-κB.[73] OipA and the cag PAI are involved in inducing interferon regulatory factor (IRF)-1 to bind and activate the ISRE-like element. Whereas CagPAI is involved in activating AP-1 and NF-κB, OipA is involved in STAT1 phosphorylation of upstream signaling of IRF-1, demonstrating that OipA and the cag PAI are both necessary for full activation of the IL-8 promoter but act via different pathways.[74] *H. pylori* induces focal adhesion kinase (FAK) phosphorylation, which in turn activates Erk, followed by actin stress fiber formation. *H. pylori* Cag A induces the phosphorylation of FAK Y407, whereas OipA adhesin phosphorylates Y397, Y576, Y577, Y861, and Y925, revealing OipA to be a better effector of stress fiber formation than CagA.[75] In addition, *H. pylori* OipA reduces IL-10 secretion and suppresses DC maturation, promoting the establishment of chronic infection.[76] The *oipA* gene is regulated by the slipped strand repair mechanism of CT dinucleotide repeats in the signal-sequence coding regions of the 5'-region. The number of CT repeats ranges from 6 to 13.[77] Isolates with different oipA CT repeat patterns, including inter-niche variation, are frequently found in a single host.[77]

HopZ, first identified by the preparation of sarkosyl-insoluble outer membrane protein, is a major adhesive, as knocking out the *hopZ* gene results in a significant decrease in the adhesive ability of bacteria.[78] Two alleles of on and off were found to regulate *hopZ* expression by slipped-strand mispairing within a CT dinucleotide repeat motif located in the signal-peptide coding region.[78] Strains harboring the *iceA1* allele, *sabA* functional status, and *hopZ* "off" status were reported to be associated with the development of MALT lymphoma, demonstrating 10 times higher odds.[79] Up-regulation of *hopZ* was observed in the transcriptional profiling of a chronic atrophic gastritis strain during infection of a mouse gastric stem cell-like line. Genetic inactivation of *hopZ* produced a fitness defect in the stomachs of gnotobiotic transgenic mice with a chronic atrophic gastritis-like phenotype, but not in wild-type littermates, implying that HopZ is important for bacterial survival in the gastric ecosystem of chronic atrophic gastritis.[80] Although the Guinea pig model showed that HopZ-deleted mutants were still able to infect the stomach, HopZ is thought to play an important role in colonization of the gastric mucosa in early infection due to the stable HopZ-ON status of isolates recovered during early infection.[81] In addition, during chronic infection, the amino acid residues of HopZ proteins are more frequently changed into ON than OFF status isolates, demonstrating *hopZ* to be one of the bacterial tools for adaptation to the host environment.

Among OMP adhesin, BabA, SabA, and AlpAB were identified to bind to blood group antigen, sialylated proteins, and laminin, respectively. However, the receptors for OipA and HopZ on gastric cells need to be investigated. In addition to physical adherence activity to the surface of gastric cells, OMP adhesins participate in or partly augment the pathogenic processes of gastric cells, including altered transcription, cytoskeletal rearrangements, disruption of cell junctions, and onset of inflammation. In the case of OipA especially, IL-8 production is articulated more frequently than adherence to the gastric epithelial surface.

## EVASION OF HOST DEFENSES

A pathogen must evade and subvert the host defense sys-

tems, including innate and T-cell immunity, and succeed in persistent colonization in host lesions. Generally, pathogenic bacteria composes pathogen-derived molecular structures (pathogen-associated molecular patterns, PAMPs) which are initially recognized by epithelial cells and innate immune cells through four distinct classes of innate immune receptors (so-called pattern recognition receptors, PRRs), including Toll-like receptors (TLRs), RIG-I-like receptors, C-type lectin-like receptors, and NOD-like receptors.[82] One of the well-defined groups of PRRs are the Toll-like receptors, of which TLR2, TLR3, TLR4, TLR5, and TLR9 have been well defined to bind to the ligands lipoteichoic acid and lipoproteins, dsRNA and polyinosinic:polycytidylic acid, LPS, flagellin, and unmethylated CpG, respectively.[83] Because *H. pylori* LPS is structurally modified, it can resist the binding of cationic antimicrobial peptides such as polymyxin B and escape detection by TLRs.[84] *H. pylori* flagellin is modified in the region corresponding to the N-terminal TLR5 recognition domain, escaping recognition by TLR5.[85] *H. pylori* DNA is intracellularly recognized by dendritic cells through endosomal TLR9, which produces a net anti-inflammatory effect, rather than a pro-inflammatory effect.[86] A unique sequence in the *H. pylori* genome, TTTAGGG has an immunoregulatory role, which is associated with alleviation of the risk of developing inflammatory bowel diseases.[87] Activation of TLR2 by *H. pylori* promotes the MyD88-dependent expression of a number of anti-inflammatory genes including IL-10. DC recognizes *H. pylori* RNA through endosomally localized TLR8 in which a cytoplasmic nucleic acid sensor RIG-I of the RIG-like helicase receptor family (RLRs) concurrently takes part. *H. pylori* also harbors ligands for the C-type lectin receptors, DC-SIGN. In contrast to the mannosylated lipoarabinomannan of *Mycobacterium*, *H. pylori*'s lipoarabinomannan is decorated with fucose residues, which actively dissociate the signaling complex downstream of DC-SIGN in the restriction of proinflammatory signaling.[88] *H. pylori* peptidoglycan is delivered to the cytoplasm by outer membrane vesicles to trigger Nod1 of the Nod-like receptor family, which induces innate and adaptive immune responses. Bone marrow DCs release caspase-1 due to *H. pylori*, which functions in the activation of not only IL-1β for driving proinflammatory response, but also IL-18 activation for balancing excessive anti-*Helicobacter* T-cell responses by restricting pathogenic Th17 responses.[89] IL-18 is crucial for the induction of regulatory T-cell responses to *H. pylori*. In the case of *M. tuberculosis*, IL-18 is depressed by antigenic stimulation.[90] There are *H. pylori* cellular factors, VacA and GGT, which promote the preferential differentiation of naive T-cells into regulatory T-cells (Tregs).[91] GGT carries a signal peptide and is rich in the sarcosine-insoluble fraction, showing that it may be located in the outer membrane rather than in the cytoplasm.[68,82,92] Tregs play a role in the suppression of *H. pylori*-specific memory T-cell responses.

## GENOMIC DIVERSITY

*H. pylori* has notoriously high chromosomal variation among isolates at the levels of both microdiversity and macrodiversity, even though proteomics display has been revealed to be nearly identical among strains.[93,94] The *H. pylori* genomic diversity includes variation in genome size, gene order, and allelic profile. About one-third of the 1600 genes predicted in the 1.6 Mbp-genome are considered to be *H. pylori* specific due to the absence of homologues in other organisms.[95] Comparison of whole genomic sequences between 2 *H. pylori* strains showed that 6–7% of each genome encode strain-specific genes, while a whole genome microarray study of 15 strains demonstrated 12–18% of each strain's genome to encode strain-specific genes.[96,97] About 22% of the genes were found to be missing in at least one of the 15 strains.[97] There is a highly variable region of 40 kb in the chromosome called the plasticity zone, in which many of the strain-specific genes showing genetic diversity among strains are located.[98,99] Genomic diversity of *H. pylori* arises through point mutation, recombination, and genetic exchange, which are supposedly frequent due to the lack of a *mutHLS* like pathway for DNA mismatch repair. DNA repeat sequences like the CT dinucleotide repeat motif frequently found in the *H. pylori* genome cause phenotypic variations, which are crucial mechanisms for immunological evasion and adaption to the gastric environment. A significant change of proteome expression was observed in strains after mouse infection.[100] In addition to intragenomic exchange of recombination, *H. pylori* can acquire foreign genetic materials from other *H. pylori* strains through DNA transformation, promoting the interstrain genetic variations observed in the *H. pylori* population. Diversification of the *H. pylori* genome is likely one of the mechanisms for persistent infection of the gastric mucosa.

## CONCLUSIONS

*H. pylori* enters the gastric cavity through contaminated in-

gesta. The gastric mucosa of humans is hostile for bacterial infection, given that gastric juice with bactericidal acidity is contained in the cavity, the mucus layer is tightly networked with continuous secretion and clearance, and host defense systems including innate and T-cell immunity are located in the submucosal lamina propria. *H. pylori* synthesizes a large amount of enzymatically potent urease to defend the bacterial cells from the gastric acidity and to focally alleviate the tightness of the mucin network to allow penetration of the mucus layer. Sheathed flagella have strong motive action for swimming in the viscoelastic mucus layer. In addition, the helical shape of *H. pylori* facilitates bacterial movement in the viscoelastic mucus layer. The tight network of the mucus layer protects the infecting bacteria from hostile factors of the luminal toxins as well as from host defense factors. A portion of the *H. pylori* swimming to the deep mucus layer adhere to the epithelial surface of the gastric mucosa through bacterial envelope structures including LPS and OMP adhesins. *H. pylori* PAMPs are structurally modified so as not to be recognized by the PRRs of innate immune cells. *H. pylori* cellular factors also promote suppression of *H. pylori*-specific T-cell responses. Active adaptation for new hosts and gastric environmental changes is accomplished by the genomic diversity of intragenomic and intergenomic changes. Conclusively, a number of host and bacterial factors are relevant for maintaining the persistence of *H. pylori* colonization in the gastric mucosa of humans, resulting in prolonged exhibition of bacterial pathogenesis, which leads to gastric symptoms. Although a number of factors are presented here, a more detailed explanation of the persistent colonization of *H. pylori* in the gastric mucosa needs to be understood sufficiently.

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