Adherence of *Helicobacter pylori* to the gastric mucosa

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M CLYNE, B DRUMM. Adherence of *Helicobacter pylori* to the gastric mucosa. Can J Gastroenterol 1997;11(3):243-248. Bacterial adhesion to the intestinal epithelium is a critical initial step in the pathogenesis of many enteric diseases. *Helicobacter pylori* is a duodenal pathogen that adheres to the gastric epithelium and causes gastritis and peptic ulceration. The mechanism by which *H pylori* causes disease has not yet been elucidated but adherence to the gastric mucosa is thought to be an important virulence determinant of the organism. What is known about adherence of *H pylori* to the gastric mucosa is summarized. Topics discussed are the mechanism of *H pylori* adherence; in vitro and in vivo models of *H pylori* infection; and adherence and potential adhesins and receptors for *H pylori*.

Key Words: *Adherence, Adhesins, Gastric mucosa,* Helicobacter pylori, *Receptors*

Adhérence d'*Helicobacter pylori* dans la muqueuse gastrique

RÉSUMÉ : L'adhérence bactérienne à l'épithélium intestinal est une étape initiale critique dans la pathogenèse de nombreuses maladies entériques. *Helicobacter pylori* est un organisme pathogène du duodénum qui adhère à l'épithélium gastrique et provoque la gastrite et l'ulcère gastro-duodénal. Le mécanisme par lequel *H. pylori* provoque la maladie n'a pas encore été élucidé, mais l'adhésion à la muqueuse gastrique serait un important facteur déterminant de la virulence de l'organisme. Ce que l'on sait au sujet de l'adhérence de *H. pylori* à la muqueuse gastrique est résumé ici. Les thèmes abordés sont le mécanisme de l'adhérence de *H. pylori*, des modèles *in vitro* et *in vivo* des modèles d'infection à *H. pylori* et l'adhérence et les adhésines et récepteurs potentiels de *H. pylori*.

Bacterial adhesion to the intestinal epithelium is a critical initial step in the pathogenesis of many enteric diseases. Binding of enteropathogens allows colonization of gut mucosal surfaces and promotes both delivery of bacterial enterotoxins and tissue invasion. Enteropathogenic *Escherichia coli* appears to induce disease by attaching to epithelial cells. Enterohemorrhagic *E coli* adheres to colonic epithelium but does not invade the epithelial cells. They produce large amounts of a toxin referred to as either shiga-like toxin or Vero toxin and are associated with watery diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans. Other organisms such as *Salmonella, Shigella* and *Yersinia* species attach to epithelial cells and subsequently become highly invasive.

Helicobacter pylori is a Gram-negative, spiral organism that adheres to gastric epithelium and causes gastritis and peptic ulceration. The method by which *H pylori* causes disease has not been elucidated but adherence to the gastric mucosa is thought to be an important virulence determinant of the organism.

ADHERENCE OF *H PYLORI* TO THE GASTRIC MUCOSA

One of the most striking characteristics of *H pylori* is that it is found in vivo only in association with gastric mucous secreting cells. *H pylori* is found within and beneath the gastric mucous layer, as well as attached to the surface of gastric epithelial cells (1). The organism is only found in the duodenum and esophagus at sites of gastric metaplasia (2) which suggests a particular tropism of the bacteria for gastric

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mucosal surfaces in humans. It is not known why *H pylori* adheres only to gastric mucus-secreting cells in vivo.

Ultrastructural analysis of gastric mucosa infected with *H pylori* shows that this bacterium causes effacement of normal gastric epithelial microvilli and closely adheres to the apical cell membrane (3). The adhesion of *H pylori* to epithelial cells is similar to that of attaching and effacing *E coli* (4-6). Attaching and effacing adherence is an intimate form of bacterial interaction with the plasma membrane of epithelial cells resulting from destruction of the microvillous cytoskeleton (7). Polymerized actin (F-actin) under regions of bacterial attachment accumulates into adhesion pedestals at the bacterium host-cell interface (8). Attaching and effacing adherence is characteristic of enteropathogenic *E coli*, and a chromosomal gene termed *eae*A gene (for *E coli* attaching and effacing) has been found to play a role in promoting adherence of enteropathogenic *E coli* to enterocytes (9,10). However, the adherence of *H pylori* appears to be different from that seen with enteropathogenic *E coli*. Dytoc et al (11) found that *H pylori* strain LC11 was unable to induce formation of F-actin adhesion pedestals on a range of eukaryotic cells. In the same study there were no F-actin adhesion pedestals found when a gastric biopsy specimen of an individual infected with *H pylori* was examined. In addition, strain LC11 stained negative by colony blot hybridization with an *E coli eae*A gene probe (11).

Adherence of enteropathogenic *E coli* to cultured epithelial cells elevates both inositol triphosphate and intracellular calcium levels (12). Elevations in inositol triphosphates also followed infection of Hep-2 cells with *H pylori* (11). Bacterial growth medium supernatants induce a similar response in HeLa cells, which indicates that inositol phosphate release in *H pylori*-infected cells is not dependent on bacterial adherence (13). In addition, the increase in inositol phosphates is not related to redistribution of cytoskeletal proteins, such as actin or alpha-actinin, or tyrosine phosphorylation of host cell proteins (13).

H pylori is generally thought to be noninvasive or to penetrate epithelial cells only rarely (3,14). In vitro intracellular uptake of *H pylori* has been shown to occur by receptor-mediated endocytosis (15). The observation of intimate contact between *H pylori* and gastric epithelial cells suggests that this may be an important feature of the disease process. Understanding the mechanism of adherence may be essential in determining how this organism causes disease.

MODELS OF *H PYLORI* INFECTION AND ADHERENCE

Experiments with laboratory animals such as rats, rabbits, guinea pigs and hamsters have shown that these are not suitable animal models for *H pylori* infection (16). The best reported animal model of *H pylori* infection is the gnotobiotic piglet (17,18). Oral inoculation of these piglets results in gastritis. *Helicobacter mustelae*, a related *Helicobacter* species, infects ferrets naturally, adheres tightly to the gastric epithelium and is associated with gastritis and peptic ulceration (19). However, experiments have failed to establish infection with *H pylori* in the ferret. *Helicobacter felis* can infect mice experimentally (20), and this model has been used to test putative vaccines for *H pylori* (21,22). Recently it has been shown that fresh clinical isolates of *H pylori* can be used to infect mice experimentally (23,24). In addition, it has been reported that *H pylori* colonizes cats naturally (25) and produces persistent gastritis in the cat stomach (26). These two recent models of *H pylori* infection may prove useful for future vaccine trials and to study the pathogenesis of *H pylori* disease.

Because of the lack of a convenient animal model in the past there has been a heavy reliance on in vitro methods to study the adherence of *H pylori*. These methods include the use of an ELISA (27), microscopy (6) and radiolabelled bacteria (4). Despite the strict tissue trophism exhibited in vivo, *H pylori* binds to a large range of cell types using in vitro methods. These cell types include human buccal epithelial cells (28), Mouse Y-1 adrenal cells (29), Hep-2 cells, Int 407 cells (6) and Kato III cells (4,30,31). However, an assay using flow cytometry to study the adherence of *H pylori* to primary epithelial cells isolated from gastric, duodenal and colonic biopsy specimens has shown that *H pylori* can exhibit the same trophism for gastric cells in vitro as it does in vivo (32).

POTENTIAL ADHESINS

Hemagglutinins: In vitro hemagglutinating activity of *H pylori* has been demonstrated with a variety of erythrocyte species (29,33-36). Different hemagglutination phenotypes have been suggested (33,34,36,37). *H pylori* has now been shown to possess at least two hemagglutinins. One has a molecular weight of 25 kDa and recognizes an N-acetylneuraminic acid moiety of receptors. The second hemagglutinin has a molecular weight of 59 kDa but the receptor specificity of this molecule is unknown (38).

The 25 kDa hemagglutinin of *H pylori* has a fibrillar morphology that recognizes specific receptors consisting of N-acetylneuraminyllactose residues in erythrocytes. This hemagglutinin has been proposed as a putative colonization factor antigen for *H pylori* (29). Treatment of erythrocytes and mouse Y-1 adrenal cells with neuraminidase inhibits adherence of *H pylori* to these cells (29,39). While treatment of Hep-2 cells with neuraminidase had no affect on the adherence of *H pylori* (40), another study reported that adherence was reduced but not abolished (15).

The present authors found that there was no correlation between expression of hemagglutinins by *H pylori* and ability of the organism to bind to either Kato III cells (cells from a gastric adenocarcinoma cell line) or to primary cells isolated from gastric biopsy specimens (32). *H pylori* was cultured on agar and in liquid broth. The method of culturing the bacteria did not affect its ability to adhere to the cells despite that hemagglutinins were not expressed in liquid culture. In addition, heating of the organism to 56°C before incubation with the cells did not affect adherence but destroyed most of the hemagglutinating activity. It is therefore unlikely that the hemagglutinins of *H pylori* play a major role in adherence to the gastric mucosa. The gene *hpa*A, which codes for the

receptor binding subunit of the N-acetylneuraminyllactose binding fibrillar hemagglutinin of *H pylori*, has been cloned and sequenced (41).

Lipopolysaccharide: Lipopolysaccharide (LPS) is a cell wall constituent of Gram-negative bacteria that is important in the structure and function of the outer membrane. LPS comprises a poly- or oligosaccharide and a lipid component termed 'lipid A' which is responsible for the toxic properties of LPS. High molecular weight smooth form LPS consists of an O side chain, which is a polymer of repeating oligosaccharide units, a core oligosaccharide unit and lipid A, whereas rough form LPS lack the O side chains. Compositional analysis of *H pylori* rough form LPS suggests that structural differences in the oligosaccharide moiety of the LPS of different strains exist (42). *H pylori* LPS has an unusual fatty acid profile (43). Moran and colleagues (42) reported that in addition to *H pylori* lipid A containing uncommonly long 3-hydroxy fatty acids, it contains an unusual phosphorylation pattern. *H pylori* lipid A exhibits low biological activity (44). Mitogenicity and pyrogenicity are about 1000-fold lower for *H pylori* LPS, and lethal toxicity is 500-fold lower compared with other enterobacterial LPS. Muotiala et al (44) suggest that the phosphorylation pattern and acylation in lipid A are responsible for the low biological activity.

Strains of *H pylori* have been reported to bind to laminin (45,46) and type IV collagen (46) in a specific and saturable manner. The interaction of *H pylori* with laminin is affected by salt, indicating that a hydrophobic component on the surface of the bacteria is involved (46). Purified LPS inhibits the binding of *H pylori* to laminin, indicating that this bacterial surface molecule is involved in the adhesion process (47,48). The interaction of *H pylori* with laminin also involves a bacterial adhesin recognising certain sialylated oligosaccharides of the glycoprotein (45). Valkonen et al (48) postulated that the initial recognition and binding of laminin by *H pylori* may occur through LPS and that subsequently a more specific interaction with a lectin-like adhesin on the bacterial surface occurs.

Outer membrane proteins: Outer membrane proteins have been shown to play a role in the adherence of *E coli* to epithelial cells (49). The outer membrane has a structural role and plays a major role in determining what enters a cell, what molecules are secreted from the cell and how the cell interacts with its environment. Little is known about the outer membrane structure of *H pylori* or the identities of its surface exposed proteins. A recent study (50) has shown that the protein content of the *H pylori* outer membrane is similar structurally to that of other *Helicobacter* species but markedly different from that of taxonomically related *Campylobacter* species and *E coli*. The outer membrane of *H pylori* NCTC 11637 contains eight major polypeptide species, at least three of which appear to be porins. Six of the outer membrane proteins have surface exposed domains. These surface exposed domains share few epitopes with related *Helicobacter* or *Campylobacter* species, which is consistent with the finding that the *H pylori* cell surface is antigenically unique. In addition, the *H pylori* LPS core displays strain to strain antigenic variability (51). These antigenic properties of the *H pylori* outer membrane surface may have implications for the ability of *H pylori* to colonize, persist and cause disease.

Urease: One of the most striking characteristics of *H pylori* is that it possesses a very potent urease enzyme. With a dissociation constant for urea of 0.8 mM (52), the urease of *H pylori* binds substrate with a much higher affinity than ureases of other bacterial species. *H pylori* and other *Helicobacter* species isolated from the gastric mucosa of animals possess a potent urease enzyme (53), whereas *Helicobacter* species that colonize the lower bowel often lack urease activity (54).

Urease is essential for colonization of experimental animals (55-57). The enzyme is thought to play a role in protecting the organism from the harmful effects of gastric acid (58,59). The urease of *Helicobacter* species differs from other bacterial ureases in that it comprises two rather than three subunits. Ure A has a molecular weight of 30 kDa and ure B, 60 kDa. A high level of homology exists among the N-terminal amino acid sequences of the enzyme subunits of four *Helicobacter* species studied by Turbett et al (53). These findings suggest a common ancestral origin and an important role for urease.

Another unusual feature of the urease enzyme of *H pylori* is that it has been located on the surface of the bacteria (60,61), which has led to speculation that the urease enzyme of *H pylori* may function as an adhesin. Fauchere and Blaser (27) reported that saline or water extracts of *H pylori* inhibited the binding of the organism to HeLa cell membranes. Gel exclusion chromatography of the extracts showed that the fractions that contained the highest urease activity bound best to HeLa cell membranes. They speculated that the adhering ligand may be urease. Gold and co-workers (62) found that a urease-deficient strain of *H mustelae* showed a selective reduction in binding to the glycosphingolipid, Gg4. When urease activity in this mutant strain spontaneously reverted during culture, wild type binding was again demonstrated.

However, several recent studies suggest that the urease enzyme of *H pylori* is unlikely to act as an adhesin. The present authors have found that a urease negative mutant of *H pylori*, strain N6(*ure*B::TnKm) – which is specifically modified in the gene that encodes for the large urease subunit, ure B, and does not produce this subunit (63) – adheres to both Kato III cells and primary epithelial cells isolated from gastric biopsy specimens, as well as the parent strain (64). Eaton and Krakowka (65) compared the ability of two isogenic mutants of *H pylori*, strain N6(*ure*B::TnKm) and strain N6(*ure*G::TnKm), to colonize gastric mucosal explants derived from neonatal germ free piglets. Strain N6(*ure*G::TnKm) contains an insertion in the *ure*G gene, one of several accessory genes necessary for expression of active urease. Both strains were able to colonize as well as the parent strain. Recently Phadnis and Dunn (66), in contrast to results from previous studies, showed that urease is a cytoplasmic protein and that there is no evidence to support secretion of urease by known bacterial secretory pathways.

They suggest that the urease of *H pylori* adsorbs to and potentially masks the outer membrane of viable *H pylori* after release by autolysis of a fraction of the bacteria.

Flagella: Motility has been shown to be an important colonization factor for *H pylori*. Nonmotile mutants of the organism are unable to colonize gnotobiotic piglets (67). A high degree of motility is conferred to *H pylori* by a bundle of three to six flagella that extend from one pole of the bacterium. The flagella consist of a basal part, which contains the flagellar motor and the hook structure, the central filament and a membranous sheath that envelops each filament (68,69). The flagellar sheath is thought to act as a protective agent against gastric acidity for the acid-labile flagellar filament (70). The major component of the *H pylori* flagellar filament is fla A flagellin (68). The filament contains a second flagellin subunit, fla B, that seems to be located mainly at the proximal part of the filament (71). Luke and Penn (72) have identified a 29 kDa flagellar sheath protein in *H pylori* using a murine monoclonal antibody. This protein was not present in the outer membrane of *H pylori*, suggesting that the flagella sheath of *H pylori* is a unique structure and not just an extension of the outer membrane. There is some evidence that for *Campylobacter jejuni* the flagellar structure may be an essential component for colonization and invasion in the host intestinal tract (73,74). Whether flagella per se are involved in attachment or solely in motility is an important question. The construction of *H pylori* (75) and *H mustelae* (76) fla A- and fla B-negative mutants and fla A-fla B double mutants (76) will greatly assist in determining whether flagella play a role in the colonization process and in mediating direct attachment of *H pylori* to gastric epithelium.

MUCOSAL RECEPTORS FOR *H PYLORI*

Phosphatidylethanolamine: *H pylori* has been shown by thin layer chromatography overlay binding assay to have a lipid binding specificity similar to that of *Pseudomonas aeruginosa*. The organism binds to phosphatidylethanolamine (PE), gangliotriaosylceramide and gangliotetraosylceramide (77,78). Considerable variation in the binding of *H pylori* to PE from different sources was observed, suggesting the importance of the nature of the long chain fatty acid. Exoenzyme S, an important adhesin of *P aeruginosa* (79), is an enzyme of *P aeruginosa* with toxic activity. It 'ADP ribosylates' several membrane-associated eucaryotic proteins. Monoclonal antibodies against exoenzyme S cross react with a 63 kDa molecule purified from *H pylori* (80). This 63 kDa molecule inhibited *H pylori* binding to PE in vitro. *H mustelae* has also been shown to bind in vitro to the same lipid receptors as *H pylori* (62). Both bovine and murine monoclonal antibodies against exoenzyme S and exoenzyme S itself inhibited the binding of *H mustelae* to PE and gangliotetraosylceramide (62). This suggests that adhesion of *H mustelae* to such species is mediated by an exoenzyme S-like molecule. The relevance of binding of *H pylori* to such molecules in vivo has not yet been studied. Construction of isogenic mutants deficient in production of the 63 kDa exoenzyme S-like molecule are required before the relevance can be resolved satisfactorily. Saitoh et al (81) showed direct binding of *H pylori* to GM3 and sulfatide using a thin layer chromatography overlay assay.

Lewis antigens: An individual's blood group antigen profile is an important factor in determining the carbohydrate structure on cell membranes. One component of this profile is the Lewis blood group antigens. Secretors express both Le^b and Le^y and A, B or H antigens in their secretions and have Le^{a-} and Le^{b+} erythrocytes. Nonsecretors express only Le^a and Le^x in their secretions and have Le^{a+} Le^{b-} erythrocytes (82).

The Lewis blood group antigens, as well as the ABO antigens, are oligosaccharide determinants attached to lipids or proteins found on epithelial cell surfaces and erythrocytes. The distribution and level of blood group antigen expression throughout various tissues of the body are variable (83-86). Using an in vitro adherence assay (87), Boren and colleagues (88) found that soluble glycoproteins representing the Le^b antigen or antibodies to the Le^b antigen inhibited bacterial binding to gastric tissue. Boren et al (88) speculated that this finding explains an epidemiological study that showed that individuals of blood type O demonstrate a higher prevalence of *H pylori* infections than those with other blood types (89).

To test the hypothesis further that the human Le^b blood group antigen functions as a receptor for *H pylori*, a human α-1,3/4 fucosyltransferase, which allows production of the Le^b antigen, was expressed in transgenic mice (90). Using an in vitro adherence assay *H pylori* bound to gastric sections from the transgenic mice, but not to gastric sections from normal mice. However, recently Balb/c and CD1 mice have been colonized by *H pylori* (23,24). The expression of Lewis blood group antigens in these animals needs to be examined to determine whether Le^b expression is required for in vivo adherence.

EXTRACELLULAR MATRIX COMPONENTS

In addition to binding laminin, *H pylori* binds to collagen type IV (46), type I (46,47) and type V (47), and to plasminogen, vitronectin, fibrinogen, fibronectin (91) and heparin sulphate (92). The relevance of such binding is unknown. It is speculated that extracellular matrix binding components help bacteria to colonize damaged tissue sites (93).

CONCLUSIONS

It is important to understand the molecular and cellular mechanisms that lead to either surface colonization or invasion of the gastrointestinal epithelium by bacteria. In addition to the ability of adhesins to attach bacteria to specific receptors on the host cell surface, adhesins also have the potential to act as biological effector molecules; adherence of organisms to a cell can trigger a cascade of events. The virulence factors and pathogenic mechanisms of *H pylori* are still not elucidated. Identification of specific adhesins and clarification of the adherence mechanism of this organism to the gastric mucosa should ultimately result in the construction of isogenic mutants. Such mutants are necessary to elucidate the mechanisms of colonization and pathogenesis of *H pylori*.

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