Virulence factors of *Helicobacter pylori*

PAUL SINCLAIR

P SINCLAIR. Virulence factors of *Helicobacter pylori*. Can J Gastroenterol 1991; 5(6):214-218. Much attention has recently been focused on *Helicobacter pylori* (formerly *Campylobacter pylori*). It is strongly implicated as the causative agent in chronic gastritis, and may be involved in gastric and duodenal ulcers, although the latter has not been confirmed. Several virulence factors have been documented, although their involvement in the pathogenesis of *H pylori* is not proven. *H pylori* would appear to have one natural reservoir, ie, the gastric mucosa of humans. To avoid this harsh environment, it is postulated that *H pylori* possesses several characteristics which enhance survival. Strong urease enzymes produced by these organism reduce urea to ammonia and appear to create a locally elevated environment with respect to pH. The spiral shape of the cells and their flagellar motility allow them to wind themselves into the mucous layer of the stomach. Some evidence exists for the production of strong proteolytic activity, hence degrading the mucous barrier and increasing permeability for the organism. Cytotoxin excreted by the bacteria may have some effect on the surrounding cells, with the possible lysis and release of bacterial growth factors. There is evidence that a chemotactic response is present due to these growth factors and their higher concentration in the intracellular spaces. The presence of specific and nonspecific adhesion has also been demonstrated, thus allowing the bacterium, once at the epithelial cell surface, to attach and avoid being washed off by movement within the stomach. Although treatment with antimicrobials eradicates the organism and improves symptoms of peptic ulcer patients, there is no indication that the same occurs in nonulcer dyspepsia patients. Further work is essential to describe the virulence mechanisms of *H pylori* and the possible pathogenic role of the organism.
(Pour résumé voir page 215)

Key Words: *Duodenal ulcers, Gastric ulcers, Gastritis,* Helicobacter pylori, *Virulence factors*

Astra Pharma Inc, Mississauga, Ontario
Correspondence and reprints: Mr Paul Sinclair, Astra Pharma Inc, 1004 Middlegate Road, Mississauga, Ontario L4Y 1M4
Received for publication May 28, 1991. Accepted November 8, 1991

THERE HAS BEEN A VAST AMOUNT of research conducted on *Helicobacter pylori* (formerly *Campylobacter pylori*) since the initial recognition of this organism in 1983 (1). Evidence has been presented in favour of the positive relationship of *H pylori* with gastritis, and gastric and duodenal ulcer disease in humans (2-4). In one study, *H pylori* was isolated from 100% of the patients presenting with chronic duodenal ulcer disease (5). Evidence for the pathogenicity of this organism also exists in the fact that two researchers have successfully infected themselves, producing typical gastritis (6,7). Additionally, eradication of the bacteria is associated with a decreased risk of relapse for duodenal ulcer (8).

Virulence may be defined generally as the ability to cause disease. *H pylori* possesses several factors which may be classified as virulence factors involved in the pathogenicity of this organism. These include motility through flagella, cytotoxin production and protease activity (9-11). The organism also produces a specific binding colonization factor antigen which is considered an important virulence factor in the

Facteurs virulents de *Helicobacter pylori*

RESUME: On accorde récemment beaucoup d'attention à *Helicobacter pylori*. Il est souvent fortement incriminé dans les gastrites chroniques et pourrait également jouer un rôle dans les ulcères gastriques et duodénaux, quoique l'hypothèse ne soit pas confirmée pour ces derniers. Plusieurs facteurs de virulence ont été relevés mais leur contribution au pouvoir pathogène de *H pylori* reste à prouver. *H pylori* semblerait avoir un réservoir naturel – la muqueuse gastrique de l'homme. Pour triompher de cet environnement hostile, on suppose que *H pylori* présente plusieurs caractéristiques qui favorisent sa survie. Ces organismes produisent une enzyme puissante, l'uréase, dont l'effet est de libérer l'ammoniac à partir de l'urée, ce qui augmenterait le pH localement. En forme de vrille et dotés de mobilité flagellaire, ils peuvent s'ancrer dans la muqueuse de l'estomac. On serait également en présence d'une forte activité protéolytique, qui dégrade la barrière muqueuse et en majore la perméabilité. Les cytotoxines excrétées par les bactéries pourraient avoir un certain effet sur les cellules environnantes, entraînant une lyse éventuelle et la production de facteurs de croissance bactérienne. Ces facteurs de croissance et leur concentration supérieure dans les espaces intracellulaires provoquent une réponse chimiotactique. Grâce à leur capacité d'adhésion spécifique et non spécifique, les bactéries parvenues à la surface de la cellule épithéliale peuvent s'y fixer et éviter d'être éliminées par le mouvement interne de l'estomac. Bien que le traitement antibactérien élimine cet organisme et soulage les symptômes de l'ulcère gastro-duodénal, rien n'indique qu'il ait le même effet sur la dyspepsie non ulcéreuse. Il est essentiel de poursuivre les travaux et de décrire les mécanismes de virulence de *H pylori* et le rôle pathogène possible de cet organisme.

colonization of the stomach and duodenum (12).

THE *H PYLORI* ORGANISM

H pylori is a Gram-negative organism usually seen microscopically as an S-shaped cell (13). This bacterium is microaerophilic, nonsporulated, motile by unipolar sheathed flagella and produces a very active urease enzyme, as well as a hemolysin, a cytotoxin and a protease (13). In vitro, *H pylori* can be grown on blood agar with growth optimal between 33°C and 40°C, with colonies visible after 72 h incubation (13). Colonies are easily identified by morphology and antimicrobial susceptibility patterns as all strains are resistant to nalidixic acid, and sensitive to cephalosporins and penicillins (14). There are several methods which can be used to detect this bacterium. As evidenced in the original discovery of *H pylori* and its association with gastric ulcers, this bacterium can be detected by various stains of biopsy specimens (1). Recently, fluorescent monoclonal antibodies to *H pylori* have been used, demonstrating high sensitivity (comparative to culture) in diagnosis from biopsy specimens (15). Several screening methods are available, such as the rapid urease test and serodiagnostic methods, but bacterial culture is favoured for the initial diagnosis of *H pylori* infection (16).

EPIDEMIOLOGY

There has been no definitive answer regarding the natural reservoir of *H pylori*. Previous bacteriological surveys of the human buccal cavity, stomach, intestine, genitourinary tract, blood and rectum have reported isolation of this bacterium in only the stomach (13). Recently, *H pylori* has been isolated from plaque and dental pulp of humans (14). *H pylori* has been found in several animal species, including monkeys, baboons and pigs (13). Interestingly, the isolation of this organism from meat handlers and veterinary surgeons is significantly higher than the general population (17). *H pylori* is found in over 50% of the world population (2,14). There is a positive correlation with the presence of *H pylori* and increasing age, and a higher prevalence is seen in certain European and Asian countries (2,14). As well, a positive correlation exists between *H pylori* and inflammatory changes in the intestinal epithelium (2). Between 90 and 100% of duodenal ulcer patients carry this bacterium (2). As well, 50 to 75% of gastric ulcer patients are positive for *H pylori* (2). Conversely, *H pylori* is isolated from 20 to 50% of asymptomatic individuals. Although there is some controversy as to the pathogenicity of *H pylori*, there is ample evidence to correlate the presence of the bacterium with the above diseases. Sufficient evidence may lie in the self-feeding experiments performed by Marshall and Morris (6,7), in which symptoms were induced by ingesting bacterial suspensions. It is thought that the bacterium alone is not capable of causing duodenal ulcers, but at least one other factor must be present for a patient to develop an ulcer. These additional factors may include genetic predisposition or environmental factors such as smoking (5).

VIRULENCE FACTORS OF *H PYLORI*

Motility: *H pylori* is a motile bacterium, propelled by four to six sheathed flagella. The S-shaped cell and the active flagella allow the organism to move within the gastric environment (13). Of particular importance is the ability of these organisms to reach the mucosal surface of the gastric epithelium from which they are commonly isolated (13). The epithelial cell layer is protected by a mucous barrier which protects against gastric acid (18). In vitro studies have demonstrated that *H pylori* rapidly penetrates viscous environments including the mucosal barrier of the gastric epithelium (18). A corkscrew mechanism is apparent as these S-shaped, flagellated cells move through viscous environments (19). An in vivo study of infection rates in gnotobiotic piglets resulted in a 100% infection rate when a highly motile strain was inoculated, whereas inoculation of a weakly motile strain produced a 40% infection rate (9). Motility correlated well as a virulence factor in colonization by *H pylori* in this animal model.

Chemotactic factors: It has been postulated that *H pylori* are attracted to the epithelial surface by chemotactic

gradients. Most bacterial cells in histological and electron microscopic sections of epithelial cell layers are closely associated with intracellular junctions of the intestinal cells (18). Serum constituents required to support the viability and growth of the bacterial cells are most readily available at this location (19). Specifically, hemin and urea are both growth factors required by *H pylori* (19). It is suggested that the bacterial cell, once closely attached to the epithelial surface, may release hemolytic factors. This hemolysin may be involved in lysis of red blood cells lying below the epithelium, releasing essential growth factors, including hemin and urea (19).

Ureolytic activity: In addition to the motility and chemotactic factors of *H pylori*, a strong urease enzyme is produced by this bacterium. In order to survive the acid environment of the stomach, the bacteria must move quickly to a more hospitable enviromnent. This is achieved, in part, by the motility and chemotactic responses. During the process of reaching the mucosal layer, and penetrating the mucous barrier, the cell produces a strong urease enzyme. One by-product of the enzymatic activity on urea is ammonia. It appears that the ammonia forms a halo locally around the bacterium, thus protecting it against the acidic conditions of the stomach (20).

Proteolytic activity: The mucous barrier of the stomach is another host barrier which *H pylori* must pass in order to reach the epithelial surface. A strong proteolytic activity towards mucus has been demonstrated, at neutral pH, in porcine gastric mucus samples (21,22). Conversely, others have reported negligible proteolytic activity in experiments performed under identical conditions, although test strains may have been subcultured several times more than in the previous study, leading to a possible decrease in proteolytic enzyme production (11). Further studies by Slomiany et al (21), demonstrated that the mucus of duodenal ulcer patients was less viscous than asymptomatic control patients. This suggests that the symptomatic patients were more susceptible to acid degradation of the epithelial cell layer due to increased permeability of the protective mucus. Although not reported, it would be interesting to correlate the presence of *H pylori* to the decreased viscosity of the mucus samples, possibly indicating an effect on mucus degradation by *H pylori*. Whether the abnormal mucus is a unique niche for *H pylori* or is a consequence of the organism's presence is a question for debate. One might propose that *H pylori* creates a local neutral environment through production of ammonia and, in addition to other in vivo conditions, may generate the reported strong proteolytic activity.

Cytotoxic activity: Cytotoxic activity has been demonstrated in culture filtrates of *H pylori* (10). This activity is heat labile and protease sensitive, indicating a proteinaceous nature. Intracellular vacuolization was demonstrated in intestinal cell cultures by transmission electron microscopy (10). Whether there is any significance to the above event in the pathogenicity of *H pylori* remains to be determined. It should be noted that all strains are capable of producing this cytotoxin in vitro, given the appropriate growth conditions. In an in vivo model of infection with *H pylori*, it was demonstrated that motile, toxigenic strains had a higher infection rate than nonmotile, nontoxigenic strains (9). Intracellular vacuolization was demonstrated in these experiments, although this event was also seen in uninfected controls. It is possible that the vacuolization is partially due to inflammatory cell mediators and normal epithelial cell turnover in the presence of the infecting organism.

Cell surface hydrophobicity: *H pylori* produces a fibrillar hemagglutinin which might be an important virulence factor. To describe this hemagglutinin adequately and the subsequent binding, it is important to review the stages prior to bacterial attachment to the epithelial surface. A recent study compared the hydrophobicity of the gastric mucosa of control and peptic ulcer disease patients (22). By microscopically measuring the contact angle of a drop of saline on dried biopsy specimens, the author was able to correlate reduced hydrophobicity in biopsies from patients with peptic ulcer disease and in *H pylori* positive patients. The significant reduction of hydrophobicity of the intestinal epithelium in patients culture positive for *H pylori* may be due to a direct effect of the presence of the bacterium, or the release of bacterial products, such as proteases and lipases, which may alter hydrophobicity (22). Ultimately, the mucosal surface appears to become more hydrophilic, and has reduced capacity to shed unnecessary material, which may include *H pylori* organisms. Similar hydrophobicity studies have been conducted on the cell surface of *H pylori*. Conflicting results have been reported regarding the cell surface characteristics of this bacterium. Using hydrophobic interaction chromatography (HIC), Embody et al (23), found that *H pylori* cells were hydrophobic. Contrasting results were produced by Smith et al (24), when they subjected strains to several hydrophobicity measuring techniques, including the HIC. An overall negatively charged cell with dominant hydrophilic characteristics was the result of this study, although distinct hydrophobic domains were also demonstrated. The disagreement in the above results is explained by the choice of hydrophobicity assay. When using the HIC method, one selects for distinct hydrophobic domains, whereas other hydrophobicity assays detect the degree of hydrophobicity of the entire bacterial cell (24). There is agreement that the binding of *H pylori* to the gastric mucosa involves specific receptor ligand complexes as well as nonspecific factors including cell surface charge and hydrophobicity (24).

Fibrillar hemagglutinin: The fibrillar hemagglutinin of *H pylori* has been described in many studies. Early studies of the ultrastructural nature of this bacterium reported a capsular-like network which surrounded cells, as demonstrated by electron micrographs of ruthenium red stained cells (25). Initial studies, aimed at demonstration of an adhesin, were developed from the standpoint that *H pylori* was involved in gastric ulcer disease, and that gastric ulcer disease was most prevalent in humans with

blood group 0 (26). As it is common for pathogens to bind to glycolipids, Lingwood et al (26), tested the binding of *H pylori* to lipid extracts of O, A and B blood cell groups and to scrapings of the pig and human stomach. A common glycerolipid was found to bind *H pylori* cells. Further studies used immunogold electron microscopy to demonstrate the fibrillar hemagglutinin (12). Electron micrographs of these cells demonstrated labelling of the entire cell surface in a capsule-like network.

The hemagglutinin of *H pylori* was sensitive to heat, papain and pronase, suggesting a proteinaceous structure. The hemagglutinin was resistant to the activity of pepsin and trypsin. These two enzymes are common in the human gastrointestinal tract, and the resistance of the adhesin may partially explain why these bacteria can survive the environment of the stomach (12). Hemagglutination inhibition assays were used to determine the exact nature of the adhesin receptor, which was found to be N-acetylneuraminyllactose. This compound is commonly found in serum proteins such as fetuin, sialoglycoproteins and sialic acid containing erythrocyte antigens (12). Similar receptor relationships have been documented for uropathogenic *Escherichia coli* and the K99 fimbrial adhesin of enterotoxigenic *E coli* (12). It is likely that N-acetylneuraminyllactose is the receptor for *H pylori* on gastric epithelial cells. Evans and colleagues (27), further substantiated their claim of receptor structure when they developed an in vitro adherence assay. *H pylori* cells adhered to mouse Y-1 adrenal cell monolayers and this adherence could be blocked by treatment of the monolayer with neuraminidase or fetuin containing neuraminlactose. Electron micrographs of Y-1 cell layers pretreated with labelled antisera to the fibrillar hemagglutinin, showed the labelled antibody between Y-1 cells and the bacterium. These three experiments confirmed the specificity of the epithelial cell receptor for *H pylori*.

To describe further the complex formed by *H pylori* cells and erythrocytes, the aggregates were viewed by transmission electron microscopy (12). The cells were seen in close contact, with an indentation visible on the erythrocyte at the point of attachment to the bacterial cell. Studies using gastric epithelial cells have demonstrated similar attachment phenomena. Three distinct adhesion categories can be seen in electron micrographs of biopsy material obtained from symptomatic ulcer patients (25). The majority of bacterial cells abut the epithelial surface, with some loss of microvilli on the epithelial cell surface. Some bacterial cells formed indentations in the epithelial cells, and others formed elevated adhesion pedestals which appeared to 'cup' the bacterium. These pedestal formations have also been demonstrated in some *E coli* intestinal pathogens (18). In vivo adherence assays using Hep-2 and Int-407 cell lines also demonstrated the cup-like structures, but no pedestal formation was detected (28). In addition to the adherence patterns observed, there was degeneration of the epithelial cells with loss of microvilli, distortion of the plasma membrane and loss of mucin granules (25). These characteristics may be due to the attachment of the bacterial cell, and subsequent release of cytotoxin or other degradative substances (25).

PATHOGENICITY IN VIVO

Several animal models have been developed to assess the pathogenicity of *H pylori* in vivo. As mentioned previously, gnotobiotic piglets were inoculated with human strains of *H pylori* (20). Stomach biopsy specimens obtained from sacrificed piglets were examined histopathologically for the presence of bacteria. The authors were successful in correlating the presence of motility and cytotoxin production with colonization of the stomach (20). They also found that the isolates recovered from the stomach were more motile than those in the inoculum. As noted before, the ability to hemagglutinate (and thus the presence of the fibrillar hemagglutinin), is absent in cultures grown in broth.

This experiment was performed using broth cultures to prepare the inoculum, therefore, a reduced virulence due to the absence of an adhesin is expected. A second small animal model was developed after a bacterial strain, similar to *H pylori*, was isolated from the stomach of a cat (29). When this strain was fed to germ free mice, similar histological changes to those in human infection were seen in the gastric epithelium. Bacteria were isolated from the mucous and deep in the intracellular spaces of the epithelium, and an acute inflammatory response was produced. In future studies, this model should prove useful in determining the pathogenicity of *H pylori*.

REFERENCES

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;ii:1311-5.
2. Tytgat GNJ. *Campylobacter pylori*: Defining a cause of gastritis and peptic ulcer disease. Scand J Gastroenterol 1988;24 (Suppl 160):59-62.
3. Lambert JR, Dunn K, Borromeo M, Korman MG, Hansky J. *Campylobacter pylori* – A role in non-ulcer dyspepsia. Scand J Gastroenterol 1988;24 (Suppl 160):7-13.
4. Smith AC. Duodenal ulcer disease: What role does *Campylobacter pylori* play? Scand J Gastroenterol 1988;24(Suppl 160):14-18.
5. Graham DY. *Campylobacter pylori* as a pathogenic factor in duodenal ulcer: The case for. Scand J Gastroenterol 1988;24:(Suppl 160):46-52.
6. Morris A, Nicholson G. Ingestion of *Campylobacter pylori* causes gastritis and raised fasting gastric pH. Am J Gastroenterol 1987;82:192-9.
7. Marshall BJ, Armstrong JA, McGehie DB, Glancy RJ. Attempt to fulfil Koch's postulates for *Campylobacter pylori*. Med J Aust 1985;142:436-9.
8. Axon ATR. *Campylobacter pylori* – Therapy review. Scand J Gastroenterol 1988;24(Suppl 160):35-8.
9. Eaton KA, Morgan DR, Krakowka S. *Campylobacter pylori* virulence factors in gnotobiotic piglets. Infect Immunol 1989;57:1119-25.
10. Leunk RD, Johnson BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. J Med Microbiol 1988;26:93-9.
11. Baxter A, Cambell CJ, Cox DM, Graham CJ, Pendlebury JE. Proteolytic activities of human *Campylobacter*

pylori and ferret gastric campylobacter-like organism. Biochem Biophy Res Comm 1989;163:1-7.
12. Evans DG, Evans DJ Jr, Moulds JJ, Graham DY. N-acetyneuraminyl-lactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: A putative colonization factor antigen. Infect Immunol 1988;56:2896-906.
13. Kasper GF. Natural sources and microbiological characteristics of *Campylobacter pylori*. Scand J Gastroenterol 1987;23:14-5.
14. Krajden S, Sherman P. *Helicobacter (Campylobacter) pylori* and acid peptic diseases. Can J Gastroenterol 1990;4:237-42.
15. Engstrand L, Pahlson C, Schwan A, Gustavsson S. Monoclonal antibodies for detection of *Campylobacter pylori* in biopsy smears and frozen sections. Scand J Gastroenterol 1987;23:50-2.
16. Megraud F. Comparison of different tests for *Campylobacter pylori*. Scand J Gastroenterol 1987;23:64-8.
17. Tytgat GNJ. *Campylobacter pylori*: Epidemiological consideration. Scand J Gastroenterol 1988;24 (Suppl 160):1-2.
18. Bode GP. Malfertheiner, Ditscheneit H. Pathogenic implications of ultra-structural findings in *Campylobacter pylori* related gastroduodenal disease. Scand J Gastroenterol 1987;23 (Suppl 132):25-39.
19. Hazell SL, Lee A, Brady L, Hennessey L. *Campylobacter pylori*dis and gastritis: Association with intracellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. J Infect Dis 1986;153:393-402.
20. Rathbone BJ, Wyatt JI, Heatly RV. Possible pathogenic pathways of *Campylobacter pylori* in gastroduodenal disease. Scand J Gastroenterol 1988;23(Suppl 142):40-3.
21. Slomiany A, Liau YH, Rosenthal WS, Slomiany BL. Sulfation of secretory and membrane glycolipids by human gastric mucosa in disease. Gastroenterology 1987;92:1644.
22. Spychal RT, Goggin PM, Marrero JM, et al. Surface hydrophobicity of gastric mucosa in peptic ulcer disease. Relationship to gastritis and *Campylobacter pylori*. Gastroenterology 1990;98:1250-4.
23. Embody L, Carlsson A. Ljungh A, Wadstrom T. Mannose-resistant hemagglutination by *Campylobacter pylori*. Scand J Infect Dis 1988;20:353-4.
24. Smith JI, Drumm B, Neumann AW, Policova Z, Sherman P. In vitro surface properties of the newly recognized pathogen *Helicobacter pylori*. Infect Immunol 1990;58:3056-9.
25. Hessey SJ, Spencer J, Wyatt G, et al. Bacterial adhesion and disease activity in *Helicobacter* associated chronic gastritis. Gut 1990;31:134-8.
26. Lingwood CA, Law H, Pellizzari A, Sherman P, Drumm B. Gastric glycerolipid as a receptor for *Campylobacter pylori*. Lancet 1989;i:238-41.
27. Evans DJ Jr, Evans DG, Graham DY. Receptor-mediated adherence of *Campylobacter pylori* to mouse Y-1 adrenal cell monolayers. Infect Immunol 1989;57:2272-8.
28. Neman-Simha V, Megraud F. In vitro model for *Campylobacter pylori* adherence properties. Infect Immunol 1988;56:3329-33.
29. Lee A, Fox JG, Otto G, Murphy J. A small animal model of human *Helicobacter pylori* active chronic gastritis. Gastroenterology 1990;99:1315-23.