Pathogenesis of *Helicobacter pylori* infection

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P SHERMAN, B GOLD. Pathogenesis of *Helicobacter pylori* infection. Can J Gastroenterol 1993;7(5):395-405. The following aspects of *Helicobacter pylori* infection are summarized: virulence factors causing *H pylori*-induced type B gastritis, the role of *H pylori* in nonulcer dyspepsia, the pathogenesis of *H pylori*-associated peptic ulceration, and mechanisms of chronic *H pylori* infection which could result in gastric carcinogenesis.

Key Words: *Adhesion, Gastric cancer,* Helicobacter pylori, *Toxin, Ulcers, Urease*

Pathogenèse de l'infection à *Helicobacter pylori*

RÉSUMÉ: Les aspects suivants de l'infection à *Helicobacter pylori* sont résumés ici: facteurs de virulence responsables de la gastrite de type B induite par *H pylori*, le rôle de *H pylori* dans la dyspepsie non ulcéreuse, la pathogenèse de l'ulcère peptique lié à *H pylori*, et les mécanismes de l'infection à *H pylori* qui pourrait évoluer vers une carcinogenèse gastrique.

THE GRAM-NEGATIVE, SPIRAL-shaped organism currently referred to as *Helicobacter pylori* (1) is established as a human pathogen causing a histopathologically evident chronic-active gastritis (2). There is specificity to the infection because other known causes of gastritis, including gastroduodenal Crohn's disease, eosinophilic gastroenteritis and drug ingestion, are not correlated with *H pylori* colonization of the antrum (3-5). Challenge of both human volunteers (6,7) and laboratory animals (8) results in the development of chronic-active gastritis. In addition, therapy clearing the bacterial infection results in resolution of the gastritis (9). Therefore, *H pylori* infection and colonization of the stomach fulfils each of Koch's postulates as a cause of chronic-active gastritis in humans (10). Accumulating evidence indicates that this organism also has an etiopathogenic role in peptic ulcer disease and gastric carcinoma, even though each of Koch's postulates have not yet been fulfilled. Several reports question whether this infection also may be a factor in the etiology of clinical symptoms in the absence of endoscopic evidence of peptic ulceration. The pathogenesis of *H pylori*-induced gastroduodenal diseases is not clearly established (11-13). Therefore, this review will highlight recent findings characterizing both the virulence properties of *H pylori* and the host responses likely involved in the pathogenesis of disease. The pathogenesis of *H pylori* infection in gastritis, nonulcer dyspepsia, peptic ulcerative disease and gastric carcinoma will be considered in sequence.

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H PYLORI-INDUCED GASTRITIS

Although it is now established that *H pylori* infection is a cause of chronic-active (type B) gastritis, the precise mechanisms involved in the induction of mucosal inflammation in the antrum are not clear. A general consideration of the factors involved in the etiopathogenesis of disease caused by other bacterial enteropathogens includes ingestion of the organism from a reservoir, colonization of mucosal sites and bacterial adhesion to mucosal surfaces, followed by either toxin production or mucosal invasion (14). Therefore, following a similar outline this review will consider the potential virulence properties of *H pylori* which

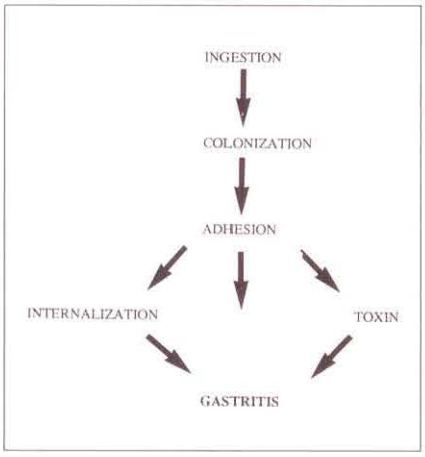

Figure 1) *Model of the stages by which* Helicobacter pylori *causes chronic-active (type B) gastritis*

may be implicated in the induction of gastritis (Figure 1).

Ingestion: As shown in Figure 1, *H pylori* must first gain access to the gastrointestinal tract. The reservoir for *H pylori* infection, however, is still not known (15). Initial studies which found that the frequency of *H pylori*-specific antibodies was higher in serum samples obtained from abattoir workers compared with controls suggested that the organism might be acquired as a zoonotic infection (16). A more recent report implicating *H pylori* as a zoonotic infection indicates seropositivity is also higher among rural Manitobans compared with their urban counterparts (17). However, an animal reservoir for this organism has not yet been identified. Concerns have also been raised about the validity of these seroepidemiological findings as there are common flagellar antigenic epitopes in both *H pylori* and enteric campylobacters. There is also evidence of intrafamilial clustering of *H pylori* infection (18) suggesting that person-to-person transmission of the gastric pathogen via either a fecal-to-oral or an oral-to-oral route might occur. The organism was recently successfully cultured from stool samples by employing selective growth media (19). Reports that dental plaque might serve as a reservoir for this gastric pathogen (20) suggest that *H pylori* might be transmitted through excretion in saliva.

Epidemiological evidence of childhood overcrowding as a risk factor for the acquisition of *H pylori* also suggests that there might be person-to-person transmission of the infection (21,22). However, another possibility is that an environmental source of *H pylori* places individuals residing within a common setting at risk for acquisition of the infection. Additional studies are clearly required to establish the environmental reservoir of *H pylori*.

Motility: *H pylori* is a flagellated organism characterized by rapid darting movements when visualized under phase-contrast microscopy (23). Recent experimental evidence suggests that motility conferred by the flagella is an important virulence factor for this bacterium. Eaton et al (24) tested nonmotile variants of *H pylori* in a gnotobiotic piglet model of *H pylori*-induced chronic gastritis. Only two of eight piglets infected with the nonmotile strain developed colonization of the stomach compared with nine of 10 piglets challenged with a motile *H pylori* isolate. Although isogenic mutants were not employed, these initial findings suggest that this organism's motility properties increase its access to both the gastric epithelium and the overlying mucous layer which then serve as the local environmental niche for long term colonization of infected humans.

Colonization: During infection in vivo *H pylori* colonizes the mucous layer overlying gastric epithelium. In vitro the organism does not survive below a pH of 2.5 (25). In vivo the organisms do not reside within the highly acidic lumen of the stomach, but rather in the more neutral pH environment formed by the mucus-bicarbonate layer immediately adjacent to the gastric epithelial surface (26).

Recent studies show that *H pylori* adheres to isolated gastric mucus in vitro. Sodium metaperiodate-induced oxidation of sugar side-chains reduces binding of mucins to *H pylori* indicating that carbohydrate side-chains on the mucus glycoprotein are involved in mediating attachment of the organism (27). Other investigators have shown that gastric mucus is degraded by urease (28,29) which is a preformed, surface-exposed enzyme that is present on the bacterial cell surface (30). Further research has shown that surface hydrophobicity in the stomach is conferred by the overlying gastric mucous layer (31-33). Measurements of the contact angle, as a determinant of surface hydrophobicity, on gastric mucosal biopsies obtained from patients with *H pylori* infection (59°) are significantly lower than on biopsies obtained from subjects without *H pylori* infection and chronic-active gastritis (70°) (34). The contact angles return towards normal values following eradication of the *H pylori* infection (35). Therefore, disruption of gastric mucus integrity by *H pylori* and its products could account for these observed changes in surface hydrophobicity. The urease enzyme could reduce the surface hydrophobicity of the infected antral mucosa by degrading mucus (28,29). One group reports that *H pylori* elaborates a protease that is also capable of degrading native gastric mucus glycoproteins (36). However, evidence of proteolytic activity in *H pylori* strains has not been confirmed by other investigators (37).

The genes encoding for production of the structural subunits of the urease enzyme, as well as regulatory and accessory proteins, have been cloned and sequenced (38-40). Use of isogenic knockout strains deficient only in the production of urease (41) should now be employed in studies to determine whether the mutants are still capable of altering the composition and functional properties of gastric mucins. As detailed below, urease has also been proposed as a virulence determinant mediating both bacterial adhesion and cytopathic effects on tissue culture epithelial cells. Therefore, isogenic urease-deficient mutants should be employed to establish whether the urease enzyme is also involved in these steps in the pathogenesis of chronic-active gastritis.

Adhesion: During infection in vivo *H pylori* also colonizes the surface of gastric epithelium, particularly in the antrum. There is electron microscopic evidence of direct adhesion between bacteria and the apical plasma membrane of the infected eukaryotic cell (42,43). Initial reports suggested a predilection of *H pylori* to intracellular

junctions; however, subsequent studies have not confirmed this observation (44). During adhesion of *H pylori* in vivo there is loss of the apical microvillus membrane and intimate contact between the surface of the organism and the apical plasma membrane of the epithelial cell (43,45). This finding has morphologic features similar to the intimate adhesion of certain types of diarrheagenic *Escherichia coli* to enterocytes and colonocytes during infection of both humans and domesticated animals in vivo and following incubation with cultured epithelial cells in vitro (46).

In vitro binding of *H pylori* to a variety of epithelial cell lines has been described including adhesion of bacteria to tissue culture cell lines derived from gastric carcinoma (eg, KATO-III cells) (47,48). During infection of KATO-III cells there is initial binding to intact microvilli and evidence of disruption of the microvillus membrane with adhesion of the infecting organism to the underlying plasma membrane (47). Recent work indicates that in vitro adhesion of *H pylori* to both KATO-III cells and differentiated epithelial cells isolated from gastric mucosal biopsies is greater than the degree of bacterial adhesion to isolated duodenal enterocytes and colonocytes (49).

During *H pylori* infection of isolated gastric epithelial cells of lapine origin there is decreased acid production (50) and increased pepsinogen release (51) from the infected cells. *H pylori* lipopolysaccharide is reported as a potential bacterial mediator inducing pepsinogen release (52). Other studies have not confirmed that there are changes in either acid production or pepsinogen release during *H pylori* infection (53,54). Thus, whether these observations correlate with changes following infection in vivo remains uncertain.

Receptor for *H pylori* binding: Previous epidemiological studies identified blood group status as a risk factor for the development of both gastritis and peptic ulcer disease; individuals with blood group O are at highest risk for these gastroduodenal lesions (55). Since blood group status is related to the sugar composition of glycolipids in the red blood cell membrane (56), in collaboration with Dr Clifford Lingwood, we sought to determine if there was specific binding of *H pylori* to lipids extracted from erythrocytes obtained from individuals of varying blood groups. Using a solid-phase partition binding assay based upon thin layer chromatograms (TLC) to evaluate *H pylori* binding to separated lipids, our hypothesis was not confirmed. TLC-overlay assays demonstrated bacterial adhesion to lipids extracted from erythrocytes of ABO blood groups (57). This study did show, however, that there is a receptor present in the lipid extract to which *H pylori* adheres. The same receptor is present in the antrum and, to a lesser extent, in the fundus of humans (57). The receptor is also present in tissue culture cell lines to which the organism attaches. Subsequently, the membrane receptor for *H pylori* binding has been characterized as a form of phosphatidylethanolamine (58). *H pylori* also binds to a deacylated form of phosphatidylethanolamine (59) and to the gangliosides ganglio-tetraosylceramide (asialo-GM-1) and gangliotriosylceramide (asialo-GM-2) (60,61). Whether these membrane receptors are surface exposed and mediate binding of *H pylori* during the infection in vivo requires further investigation.

Adhesins expressed by *H pylori*: The bacterial product mediating adhesion of *H pylori* to gastric mucus and to gastric epithelial surfaces is not yet known. It is likely, however, that multiple determinants are involved in binding of the organism to surface receptors. In a superb review of bacterial adhesion by Hasty et al (62) it was concluded that "bacteria also express multiple adhesins and further, that the adhesins probably function in distinct kinetic steps". The same review indicates that there is "no need to bring attention to the concept of multiple adhesins in Gram-negative bacteria so widespread is its acceptance". It is likely that this concept also holds true when considering *H pylori* adhesion to gastric epithelia both in vitro and in vivo. However, knockout mutations of potential adhesin genes in the organism will be required to definitely establish whether a single virulence determinant or multiple genes encode the adhesins which promote binding of *H pylori* to mucosal surfaces.

H pylori strains are known to agglutinate erythrocytes (63-65). Hemagglutination indicates that there is a receptor on the plasma membrane of red blood cells. It also demonstrates that a hemagglutinin expressed on the bacterial cell surface mediates attachment. However, this mannose-resistant hemagglutination may not correlate with binding of *H pylori* to gastric epithelial cells and gastric mucus. One group has reported that *H pylori* expresses a hemagglutinin that binds to a specific lactose-containing receptor and that this bacterial product also might be involved in binding the organism to epithelial surfaces (66). This hemagglutinin is expressed in vivo because there is a specific humoral response in infected individuals (67). Another group has reported that some *H pylori* strains elaborate a soluble hemagglutinin that is a potential adhesive factor (68). However, this finding has not been duplicated in other laboratories.

Phosphatidylethanolamine, to which *H pylori* adheres, also serves as a receptor for binding of exoenzyme S expressed by *Pseudomonas aeruginosa* (69). *H pylori* expresses a similar protein, referred to as exo H, which may serve as a ligand for attachment of this organism to lipid receptors (58). There is one study which described the identification, cloning and sequencing of a gene encoding for a non-fimbrial surface-exposed fibrillar protein on the surface of bacteria which may function as an adhesin (70). Other studies suggest that the urease enzyme might modulate *H pylori* adhesion or serve as a bacterial ligand (71). Flagella are described as adhesins for closely related enteric campylobacters (72). Therefore, the potential role of flagella as binding proteins for *Helicobacter* species should be investigated further.

For other gastroenteric pathogens, surface hydrophobicity plays a role in the initial establishment of contact between the bacteria and mucosal surfaces (73). Studies evaluating the surface hydrophobicity properties of *H pylori* have yielded varying results (74,75). These differences may be accounted for by the use of different bac-

TABLE 1
Immune responses of the host to *Helicobacter pylori* infection

B cell	Increases in IgG, IgA, IgM
T cell	Increases in IL-1, IL-2, IL-2 receptor
Macrophage	Increases in TNF-α, IL-8
Epithelial cells	Expression of MHC class II antigens, increases in IP3, IL-8
Polymorphonuclear leukocytes	Possible effect on nitrate production

Ig Immunoglobulin; IL Interleukin; IP Inositol tris-phosphate; MHC Major histocompatibility complex; TNF Tumour necrosis factor

TABLE 2
Proinflammatory products of *Helicobacter pylori*

Urease
Lipopolysaccharide
Heat shock protein
Platelet-activity factor
Phospholipase A_2
Phospholipase C
Catalase

terial strains and variable growth conditions. It is also possible that specific hydrophobic domains, which have not yet been identified, mediate initial adhesion of the organisms to gastric epithelial surfaces (75).

Toxin production by *H pylori*: Incubation of both *H pylori* and cell-free culture supernatants result in cytopathic effects in a variety of eukaryotic cells grown in tissue culture (76). It was reported that these cytopathic changes could simply relate to the potent urease activity of *H pylori* (77). Conversely, more recent studies suggest that there is a distinct cytotoxin expressed by *H pylori* strains that induces formation of autophagocytic lysosomes (78). The cytotoxin structural subunit, with a molecular mass of 85 kDa, has been purified and sequenced (79). The toxin is expressed during infection in vivo because antibodies to the toxin develop in *H pylori* colonized individuals (80,81). In addition, one study reported that there is a greater frequency of identifying *H pylori* isolates producing cytopathic effects in vitro in individuals with peptic ulcer disease compared with subjects with gastritis alone (82). This provocative finding requires confirmation at other centres. Isogenic mutants deficient in toxin production are required to define more precisely the role of toxins in *H pylori*-induced gastroduodenal diseases.

Invasion of *H pylori*: Most histopathological studies of infected humans have not provided evidence of bacterial invasion into the cytoplasm of surface gastric epithelial cells or the underlying interstitium. However, *H pylori* binds to extracellular matrix proteins in vitro (83) and there are isolated reports identifying the organism within the lamina propria of gastric epithelium (84). Following *H pylori* infection of cultured epithelial cells, one group reports that there is an internalization of intact, viable organisms into the cytoplasm of the eukaryotic cell (85). Whether *H pylori* is internalized by gastric epithelial cells requires investigation. The relevance of these in vitro findings to infection in vivo also must be defined. Genes coding for this property of internalization of the organisms into susceptible epithelial cells should be identified to confirm these initial laboratory observations.

Immune responses of the host: During *H pylori* infection of humans there is the development of a chronic-active gastritis (2,3). In contrast, in experimental animal models there is evidence of chronic inflammation but in most models there is no evidence of an acute inflammatory response (8). This suggests that most animal models do not provide an accurate reflection of the inflammatory responses in infected humans. Further development and detailed characterization of more appropriate animal models of *H pylori* infection are, therefore, urgently required.

As shown in Table 1, during *H pylori* infection of humans there is evidence of infiltration into the gastric epithelium of T lymphocytes, B cells, plasma cells, macrophages and polymorphonuclear leukocytes (86). There is both immunohistochemical and immunoassay evidence of increased levels of immunoglobulins G, M and A production in the local gastric microenvironment (87). However, despite the vigorous humoral and T cell immune responses there is little evidence to suggest spontaneous clearing of the organism once colonization and adhesion to gastric mucosal surfaces have developed.

The gastritis caused by *H pylori* infection is associated with the induction of major histocompatibility complex (MHC) class II antigen expression on the surface membrane of gastric epithelial cells (88). MHC class II antigen expression also occurs in epithelial cells in other areas of the gastrointestinal tract during acute and chronic inflammation (89). This could have important etiopathological relevance since MHC class II molecules are involved in the host mucosal immune response. In addition, murine monoclonal antibodies raised against *H pylori* cross-react with antigens exposed on the surface of mucus-producing cells in the gastric antrum of infected individuals (90). Cross-reacting antibodies are also present in the serum of *H pylori*-infected individuals but not in uninfected controls (91).

H pylori infection also induces infiltration of both macrophages and neutrophils into the gastric mucosa. In vitro evidence indicates that these proinflammatory cells are activated. For example, Mai et al (92) showed that chemiluminescence of both monocytes and neutrophils is increased, in a dose-dependent fashion, by cell-free culture supernatants of *H pylori*. This chemiluminescence response is significantly reduced by coincubation of the bacterial supernatants with anti-urease antibodies (93). This suggests that the urease enzyme also acts as a proinflammatory agent. As shown in Table 2, other bacterial constituents which might initiate the proinflammatory cascade include bacterial heat-shock proteins (94,95), platelet activating factor (96), phospholipase A (97), phospholipase C (98), catalase (99), and lipopolysaccharide (LPS). LPS extracted and purified

from *H pylori* has a structural composition that is distinct from other Gram-negative organisms (100,101) and low biological activity demonstrated with a standard limulus lysate assay (102,103). However, the biological effects of *H pylori* LPS on the gastric microenvironment and on gastric epithelial cells may differ from these in vitro assay results. Young et al (52) showed, for example, that pepsinogen release from epithelial cells is activated to a greater degree by LPS derived from *H pylori* than LPS extracted from other Gram-negative bacteria.

The gastric inflammatory response to *H pylori* infection results in the release of a number of cytokines and lymphokines including tumour necrosis factor-alpha, interleukin-1, interleukin-6 and interleukin-8 (104-106). This proinflammatory cascade ultimately results in the chronic-active inflammation that is characteristic of *H pylori*-induced gastritis. A current working hypothesis proposed by Blaser (107) suggests that by inducing this cascade the organism derives nutrients from the host for continued colonization and replication. *H pylori* infection also induces a signal transduction response within epithelial cells. In a preliminary study we showed that inositol triphosphate levels are elevated in the cytosol of infected tissue culture epithelial cells compared with cells grown in the presence of heterologous organisms or without bacterial infection (43). The reason(s) why these inflammatory, immune and signal transduction responses do not successfully clear *H pylori* colonization requires explanation because it could provide information critical to the development of novel strategies to either prevent or eradicate *H pylori* infection.

PATHOGENESIS OF *H PYLORI*-ASSOCIATED NONULCER DYSPEPSIA

Current evidence does not support an etiopathogenic role for *H pylori* infection as a cause of clinical symptoms in the absence of active peptic ulceration (108). Most studies evaluating adults with nonulcer dyspepsia do not provide evidence of an increased rate of infection compared with controls (109). Age-matched controls are essential in these epidemiological studies as there is an age-dependent increase of asymptomatic *H pylori* infection (110, 111). In addition, one study demonstrated that the clinical response following therapy eradicating *H pylori* is not significantly better compared with the use of placebo (112).

H pylori seropositivity rates are not higher in children with functional recurrent abdominal pain compared with the prevalence of infection among age-matched controls (113,114). Our group was unable to identify a specific clinical symptom, or symptom complex, in the evaluation of children undergoing diagnostic upper endoscopy for symptoms of dyspepsia. In this study there were no factors that clearly segregated individuals with documented *H pylori* infection from symptomatic patients without bacterial colonization of the antrum (115). In summary, there is little current evidence to suggest that in the absence of peptic ulceration *H pylori*-induced gastritis is a cause of clinical symptoms.

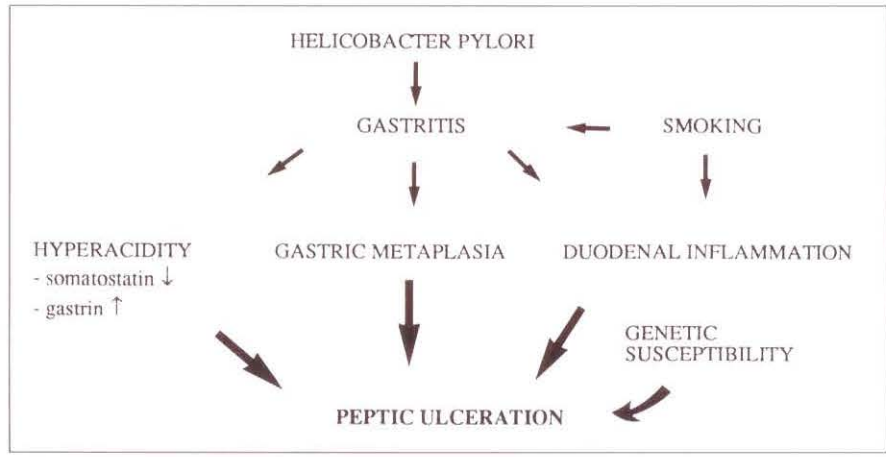

Figure 2) *Mechanisms postulated for* Helicobacter pylori*-induced peptic ulceration*

H PYLORI-ASSOCIATED PEPTIC ULCER DISEASE

Epidemiological studies identify a strong association between the presence of *H pylori* infection in the antrum and duodenal ulcer disease (9). The epidemiological association of *H pylori* infection with gastric ulcer is less certain. The most convincing evidence demonstrating that *H pylori* colonization of the antrum is causally related to peptic ulcer disease are studies which show that the relapse rate following healing of peptic ulcers is dramatically altered by eradicating *H pylori* infection (116). The 80% one-year recurrence rate for peptic ulcers following standard ulcer-healing therapy is reduced to under 20% for both duodenal ulcers and gastric ulcers (117). These studies provide compelling evidence, therefore, that *H pylori* infection is indeed related to gastroduodenal ulcer disease. In a recent excellent critical review of the potential role of *H pylori* in peptic ulceration, Moss and Calam (118) indicate that "all but the most perverse now accept the abundant evidence that *H pylori* plays an important role in relapse of duodenal ulcer disease" and that "both marketing and scientific curiosity call for studies to determine how *H pylori* does, or in most individuals does not, cause peptic ulcers".

It must be emphasized, however, that *H pylori* infection does not fulfil each of Koch's postulates as a cause of peptic ulcer disease. Infected human volunteers develop chronic-active gastritis but not peptic ulceration (5,6). Similarly, duodenal ulcers have not been described in the various animal models of helicobacter infection (7). Nevertheless, the accumulating and compelling evidence that eradication of *H pylori* infection results in a change in the natural history of recurrence of peptic ulceration raises questions regarding the etiopathogenesis of bacterial infection in the stomach and

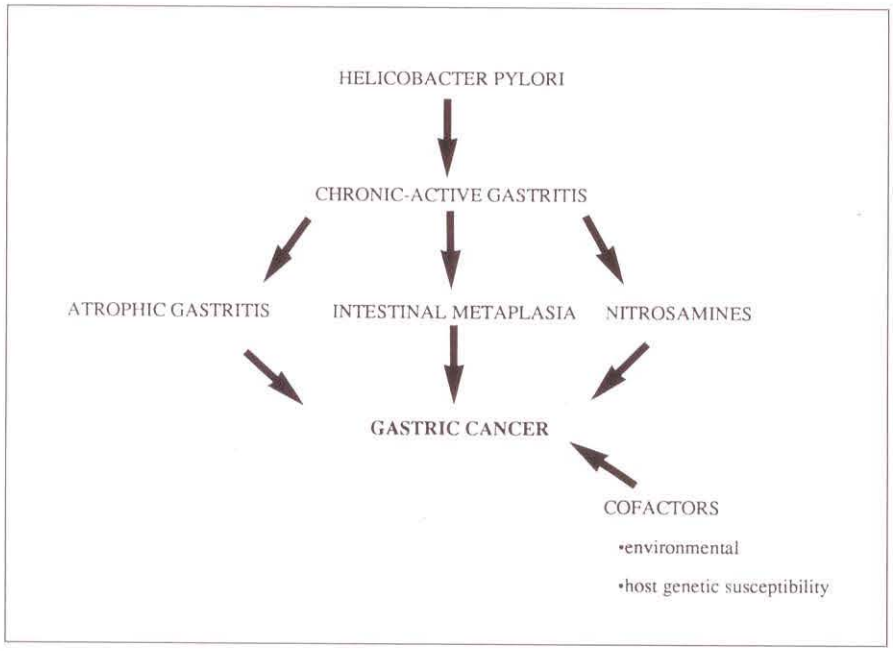

Figure 3) *Possible pathways by which chronic* Helicobacter pylori *infection results in gastric cancers*

associated peptic ulcer disease. Possible mechanisms for *H pylori*-induced ulcerogenesis (Figure 2) are considered below.

Gastrin: Levy et al (119) reported that meal-stimulated gastrin levels are elevated in patients with *H pylori* infection and duodenal ulcer disease. This exaggerated postprandial gastrin response, related primarily to gastrin-17 (120), is reduced towards normal following eradication of *H pylori* infection (121). An exaggerated response following meals is also identified among patients with *H pylori* infection in the absence of an ulcer diathesis (122). It has been suggested, therefore, that *H pylori* colonization of the antrum, and associated bacterial urease activity, results in a locally inappropriate elevation of luminal pH (123). Gastric G cells sense that there is too little acid in the gastric lumen and thereby produce increased amounts of gastrin in response to a meal. Increased gastrin could then result in postprandial excesses of acid and pepsinogen delivered into the first part of the duodenum and thereby induce peptic ulceration in the susceptible individual. This attractive hypothesis, however, has not been confirmed since other investigators have been unable to document a consistent increase in postprandial gastrin (124,125) or specific mRNA (126) levels.

Somatostatin: Other investigators suggest that the postprandial excess in acid production following *H pylori* infection may not be related to excessive amounts of gastrin, but rather due to decreased amounts of somatostatin (127,128). Recent studies suggest that the amount of specific messenger RNA for somatostatin and somatostatin product translated by D cells in the stomach are both reduced in individuals with *H pylori* infection. Moreover, somatostatin transcription and translation products increase following eradication of *H pylori* infection (126). Since somatostatin release is involved in the control of acid production (129,130), changes occurring with bacterial colonization of the antrum could result in excess delivery of acid into the duodenum and thereby promote ulcerogenesis in susceptible individuals.

Metaplasia: An alternate explanation for *H pylori*-induced duodenal ulcer is that the organism colonizes inflamed mucosa in the duodenum. Wyatt et al (131) showed that metaplasia is more frequent among individuals with *H pylori* infection and that duodenal ulcers frequently occur in the duodenum immediately adjacent to the foci of gastric metaplasia. We have reported similar findings in children with *H pylori* infection (132). The frequency of metaplasia in the first part of the duodenum was significantly higher in *H pylori*-infected children compared with age-matched subjects with gastritis due to causes other than *H pylori* infection or among patients without gastritis.

Duodenitis: Whether metaplasia in the duodenum is a risk factor for duodenal ulceration is not entirely clear because it is also associated with an active duodenitis (131-133). Duodenal inflammation is also more frequent among individuals with *H pylori* infection of the antrum compared with individuals with secondary causes of gastritis or among subjects without evidence of gastritis (132). Longstanding mucosal inflammation may predispose the duodenum to active erosions and ulcerations in susceptible individuals. Release of the bacterial proinflammatory products listed in Table 2 into the duodenum could also induce proinflammatory responses in the proximal small bowel.

Cofactors: Additional risk factors such as smoking and reduced local cytoprotective effects, including reduced mucosal bicarbonate production in the first part of the duodenum (134), are also likely to be involved in determining which individuals will develop peptic ulceration. Genetic susceptibility and environmental risk factors, such as smoking, may ultimately determine which individuals among the large number of subjects with *H pylori* infection ultimately will develop peptic ulcers. It should be emphasized, however, that smoking is a risk factor for ulceration only in the presence of *H pylori* infection (135). The risk of recurrent ulceration with continued abuse of tobacco is evident only among subjects in whom the associated *H pylori* infection is not successfully eradicated (135,136).

In summary, with the evidence currently available it appears that *H pylori* infection in the antrum is indeed related to peptic ulcer disease. Whether increases in postprandial gastrin, decreases in mucosal somatostatin production, gastric metaplasia in the duodenum, or chronic duodenal inflammation are primarily responsible for ulcerogenesis requires further evalu-

ation. An animal model of helicobacter infection in which there is reproducible induction of duodenal ulcers would greatly facilitate this avenue of investigation.

H PYLORI AND GASTRIC CARCINOMA

Epidemiological studies have described an association between *H pylori* infection and the development of a variety of types of gastric cancer (137-139). Although epidemiological studies only identify an association and do not define cause and effect, they suggest that longstanding *H pylori* infection, particularly if acquired during the childhood years (140), is an environmental risk factor for the development of gastric carcinoma. If confirmed, removing this risk factor could dramatically alter the significant morbidity and mortality associated with the development of gastric cancers (139).

The question of how longstanding *H pylori* infection ultimately results in carcinogenesis is not well understood. As summarized in Figure 3, it has been proposed that longstanding inflammation in the stomach induces atrophy in the antrum and intestinal metaplasia in the body of the stomach; both events are reported to precede gastric cancer (141,142). A recent study evaluating colonic carcinoma showed that nitrate production by neutrophils infiltrating into the local mucosal environment is a potential risk factor for carcinogenesis (143). Since neutrophilic infiltration is a histopathological feature of chronic-active gastritis in humans with *H pylori* infection, it is of considerable interest to now determine whether neutrophils derived from gastric epithelium are also capable of forming procarcinogenic agents such as nitrates. In addition, the urease activity of *H pylori* may also be a factor in promoting gastric malignancy (144).

CONCLUSIONS

There has been a rapid explosion of information about the virulence properties of *H pylori* since the initial culture and identification of this organism by Marshall and Warren some 10 years ago. However, as shown in Table 3, there remain a series of important unanswered questions. These include an understanding of the remarkable tissue tropism, the narrow host range and the marked genetic heterogeneity of *H pylori* (145,146).

As summarized in Table 4, to better understand the virulence properties of *H pylori* it is important to now determine whether there are additional virulence factors expressed by the organism involved in the induction of chronic-active gastritis, duodenal ulceration and gastric carcinoma. The relative importance of the virulence factors identified to date, and summarized in this review, requires further evaluation. The dissection of the relative importance of *H pylori* virulence determinants ultimately will be undertaken by improving our understanding of the genetic basis and genetic control of the virulence properties of *H pylori*. The identification, cloning and sequencing of additional genes within the *H pylori* genome and a determination of those genes contributing to the virulence of the organism are required. Successful identification and sequencing of urease genes in *H pylori* and the creation of isogenic mutants indicate that the genetics of *H pylori* infection will be a rapidly advancing field of investigation over the next several years.

Since normal immune and inflammatory responses are not sufficient to enable successful clearing of this infection once it has colonized the antrum, the host responses to bacterial infection also are a priority area of future investigation. An explanation of the lack of effective bacterial clearing could provide important insights into understanding the pathogenesis of this bacterial disease. In addition, novel therapeutic options may become evident. For example, the development of vaccines to prevent both recurrence of *H pylori* infection among successfully treated individuals and high risk uninfected populations should become possible with an improved understanding of the etiopathogenesis of disease. If bacterial adhesion is an essential virulence property, then defining the adhesin, or adhesins, could result in the development of effective passive and active vaccine candidates. Such options are available for enteric infections in domesticated animals (147) and are in a rapid phase of development for enteric bacterial infections in humans (148).

Recent reports indicate that it will be possible to develop novel vaccines for preventing *H pylori* infection (149). Studies to date have employed an animal model to show that, in conjunction with small doses of cholera toxin given as adjuvant (150), an effective immune response develops which prevents bacterial colonization during subsequent oral challenge (151,152). These initial findings hold out promise that safe and effective vaccines for use in the prevention of gastroduodenal diseases will become available in the future for therapeutic applications in humans.

TABLE 3
Pathogenesis of *Helicobacter pylori* infection: Unanswered questions

Environmental reservoir(s)
Tissue tropism
Narrow host range
Strain variation
Relative importance of 'known' virulence factors

TABLE 4
Virulence properties of *Helicobacter pylori*: Future directions

Genetic understanding of virulence
Identification of virulence genes
Isogenic deletion of virulence genes
Host immune responses to infection
Host inflammatory response to infection
Animal models of infection with peptic ulceration
Progression to malignancy
Vaccine development

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