Understanding disease outcome following acquisition of *Helicobacter pylori* infection during childhood

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AS Day, PM Sherman. Understanding disease outcome following acquisition of *Helicobacter pylori* infection during childhood. Can J Gastroenterol;13(3):229-234. *Helicobacter pylori* causes chronic active (type B) gastritis in the overwhelming majority of infected individuals. The relative contribution of virulence factors in the bacterium and host responses to the microbial infection in determining which subjects will go on to develop complications – such as peptic ulceration, gastric cancers and gastric lymphomas – is the subject of current investigative activities.

Key Words: *Children, Cytotoxin, Gastric cancer,* Helicobacter pylori, *Metaplasia, Ulcers*

Comprendre le pronostic de la maladie après une infection à *Helicobacter pylori* acquise pendant l'enfance

RÉSUMÉ : *Helicobacter pylori* cause une gastrite chronique et évolutive (type B) chez la grande majorité des sujets infectés. La contribution relative des facteurs de virulence de la bactérie et de la réponse de l'hôte à l'infection microbienne à déterminer quels sujets développeront des complications – telles qu'ulcérations gastro-duodénales, cancers et lymphomes gastriques – est le sujet des recherches actuelles.

Helicobacter pylori infection generally is acquired during early childhood (1) when it induces a chronic active type B gastritis. Risk factors for infection early in children include residing in developing countries (2) or urban settings (3), and growing up under poor socioeconomic circumstances (4). The latter risk factor may reflect enhanced exposure to a common, but as yet unidentified, environmental reservoir of the organism or heightened person to person transmission due to family crowding occurring as a result of financial deprivation (5). The increased prevalence of gastric infection among children cared for in institutional settings (6) supports the contention that humans serve as a vehicle for transmitting the organism.

Unless eradication therapy is introduced, colonization of the antrum of the stomach by *H pylori* and the accompanying mucosal inflammation generally persist over the course of a lifetime. Most infections remain asymptomatic, with only a small subset of infected individuals developing complications including peptic ulcer disease and gastric cancers. Fennerty (7) estimated that the lifetime risk of developing a gastric ulcer or duodenal ulcer as a complication of *H pylori* infection is about 15%. The risk of developing a gastric adenocarinoma was estimated as 0.1% of all *H pylori*-infected persons. The estimated risk of *H pylori* causing a gastric lymphoma was even lower – 0.001% (7).

There is an ongoing controversy regarding the merits of instituting eradication therapy in all individuals who are infected with *H pylori* (8). This question could be clarified if an accurate method were available to predict which subjects are at greatest risk for the development of significant complications of chronic gastric infection including peptic ulceration, adenocarcinomas involving the antrum and body of the stomach, and gastric lymphomas. Such an approach would focus attention and resources on the treatment of infection in high risk individuals. Persons at low-risk of the complications of *H pylori* infection would avoid the side effects of eradication therapy while minimizing financial costs to the health care system.

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TABLE 1
Factors that may identify ulcerogenic *Helicobacter pylori*

Vacuolating cytotoxin (VacA)
Cytotoxin-associated outer membrane protein (CagA)
Pathogenicity island (*cagE*/*picB*)
Invasion of epithelial cells

There is no accurate way to discriminate the person infected with *H pylori* who is at risk for developing peptic ulcer disease from the large number of people infected with the organism who will remain asymptomatic. An interesting report by Hansson et al (9) suggests that infected humans who develop duodenal ulcer disease are distinct from those who develop gastric cancers. The question is whether this evidence points towards the possibility of ulcerogenic and carcinogenic strains of *H pylori*. Alternatively, all *H pylori* strains may be equally virulent, but the disease manifestations may reflect host responses to the chronic infection (10).

This critical review considers what is currently known about the bacterial and host factors that may play a role in the development of peptic ulcer disease as a complication of *H pylori* infection.

PREDICTING ULCER DISEASE AS A COMPLICATION OF *H PYLORI* INFECTION

There is compelling evidence that *H pylori* infection causes both gastric ulcers and duodenal ulcers. Eradication of infection enhances ulcer healing (11) and resolution of clinical symptoms. The natural history of peptic ulcers to recur following healing with acid suppression alone is dramatically altered by eradication of *H pylori* colonization in the antrum of the stomach (12). Clinically, the reduction in ulcer recurrence from 50% or more with acid suppression alone to less than 5% at one year of follow-up with eradication therapy is extremely relevant because the risk of recurrent bleeding – with its attendant morbidity and mortality – is also markedly reduced (13).

Discriminating among strains of *H pylori* requires the development of methodologies to categorize the bacterium into subgroups. Typing systems are employed to identify bacterial isolates that are identical. Such information can be very helpful in identifying common outbreaks. A variety of methods have been employed with success in other Gram-negative pathogens. For example, serotyping systems have been developed to distinguish between commensal strains and those causing clinical disease (14). Serotyping can be based on antigenic variation in heat-labile antigens (such as flagella and outer membrane proteins) and heat stable lipopolysaccharide-derived antigens. An initial report suggested that monoclonal antibodies directed against the various Lewis antigens expressed by *H pylori* lipopolysaccharide (15) could be employed as a serotyping method to subcategorize clinical isolates. However, subsequent studies have shown that the expression of the antigens detected by these monoclonal antibodies is subject to phase variation under growth conditions employed in the laboratory (16). Therefore, the lipopolysaccharide-based typing system has not been employed to discriminate successfully *H pylori* strains isolated from patients with peptic ulcer disease from those with chronic active gastritis alone.

Susceptibility profiles to antibiotics have been used with success to identify common sources of outbreaks of infection caused by other Gram-negative bacteria (17). However, the rapid change in antibiotic susceptibility of *H pylori* in response to environmental exposure makes this an unsuitable approach to discriminate bacterial strains likely to induce peptic ulcer disease from those isolates that cause gastritis alone.

A genetic approach to the categorization of microbial pathogens has been employed successfully for a variety of Gram-negative bacteria infecting the intestinal tract. The marked genetic heterogeneity of *H pylori* infecting the stomach has made this a less than successful approach to date. DNA fingerprinting of *H pylori* isolates reveals such a remarkable heterogeneity that every individual appears to be infected with a different bacterial strain (18). Among strains causing comparable disease outcomes, such heterogeneity complicates the development of an accurate typing system based on genetic similarity. Plasmid profiles have not been reported as a successful marker for use in the subtyping of *H pylori*.

In summary, there is currently no suitable marker to distinguish *H pylori* isolated from subjects with peptic ulcer disease from those with gastritis alone by using phenotypic and genotypic typing systems that are traditionally employed for Gram-negative enteropathogens. This deficiency has led investigators to consider whether virulence factors only present in bacteria isolated from patients with disease complications might be employed in an effort to provide a method to identify ulcerogenic *H pylori* isolates (Table 1).

VIRULENCE FACTORS OF *H PYLORI*

Vacuolating cytotoxin: Many *H pylori* isolates produce a toxin that causes cytopathic effects on a variety of cell lines grown in tissue culture (19). The toxin exerts its effects through the low molecular weight G protein Rab7 (20) by disrupting trafficking in the late endosome compartment of infected eukaryotic cells (21). The role of the vacuolating cytotoxin (VacA) in vivo is still uncertain because most investigators do not describe vacuolization of gastric and duodenal epithelial cells as a histopathological feature of the infection. Nevertheless, some reports indicate the presence of vacuolated cells in vivo in subjects colonized with an *H pylori* strain that produces VacA (22).

Regardless of its biological relevance, VacA may also serve as a marker of virulence. Indeed, some studies suggest that VacA strains are isolated with a higher frequency among subjects with *H pylori*-associated peptic ulcer disease than in patients with *H pylori*-induced gastritis alone (23,24). Unfortunately, these initial reports have not been

confirmed in subsequent studies by other investigators (25). The presence of the VacA phenotype in *H pylori* strains isolated from children and adolescents does not discriminate between those with duodenal ulceration or duodenitis and those with gastritis alone (26,27).

Allelic variations in the gene encoding VacA have been proposed as a more sensitive marker than the cytotoxic phenotype for discriminating between ulcerogenic and non-ulcer-related *H pylori* isolates. Variations in the signal sequence, ie, s1a variants in the *vac*A gene, are reported to be present more commonly in patients with duodenal ulceration than in those with gastritis alone (28,29). These findings require confirmation in a variety of community settings because not all investigators have been able to show that alleles of *vac*A serve as useful predictors of disease outcome (30,31).

Cytotoxin-associated outer membrane protein: A gene first identified in association with *vac*A, and, therefore, referred to as the cytotoxin-associated gene (*cag*A), may also serve as a marker of bacterial virulence. The gene codes for a 128 kDa outer membrane protein. Cover et al (32) reported the presence of the gene in 82% of *H pylori* isolates obtained from patients with duodenal ulcer disease versus only 59% of 39 subjects with gastritis alone. Similarly, immune responses to CagA occur more frequently in *H pylori*-infected persons with duodenal ulceration than in those with gastritis alone (32,33). However, this discriminatory power is true only in North American and European studies. By contrast, infection with CagA-producing *H pylori* is the norm in China, Japan and other countries in the Far East, regardless of the presence or absence of peptic ulcer disease as a complication of infection (34,35).

Allelic variations in *cag*A may serve as a better marker of disease outcome, but further studies in both communities at high risk and at low risk for the development of disease complications are required. For example, there is increasing interest in determining why the frequency of peptic ulceration and bleeding complications is so low in persons living in Africa despite a very high prevalence of *H pylori* infection (36). Studies evaluating the *cag*A variants present in *H pylori* strains isolated in Africa compared with those isolated in North America and Western Europe are awaited with interest.

PATHOGENICITY ISLAND

Unlike *vac*A, *cag*A resides on a 38 kilobase segment of chromosomal DNA that comprises a guanine plus cytosine content (35%) that is lower than that of the rest of the bacterial genome (39%) (37). This suggests that *cag*A is part of a pathogenicity island, which, in other bacterial pathogens, often encodes virulence properties (38).

The *cag* pathogenicity island contains a number of open reading frames that, as deduced by their similarity to related genes in other Gram-negative bacteria, encode a type IV secretion pathway (39). Increasingly, it is recognized that prokaryotes can use a variety of secretion pathways as a means of delivering bacterial products to infected eukaryotic cells (40). It is possible, therefore, that protein products of the *cag* pathogenicity island in *H pylori* are delivered into infected epithelial cells and, thereby, influence disease outcome.

Current interest focuses on the role of *cagE* (also referred to as *picB*) because the presence of this gene correlates with *H pylori* isolates that induce a larger chemokine and resulting proinflammatory response in epithelial cells infected in vitro (41). Whether other gene products of the pathogenicity island correlate with the presence of peptic ulceration and other complications of *H pylori* infection is the subject of current investigation (42).

TABLE 2
Host factors that may predispose *H pylori*-infected individuals to duodenal ulceration

Increased pepsinogen
Increased acid
Enhanced gastrin
Reduced somatostatin
Altered motility
Ectopic gastric mucosa
Duodenitis
Increased interleukin-8
Increased tumour necrosis factor-alpha
Increased interleukin-6

HOST RESPONSES TO *H PYLORI* INFECTION

Are all *H pylori* strains capable of causing peptic ulceration? Some studies suggest that this may be the case. For example, some reports indicate that there is a higher bacterial load in the stomach of patients with documented duodenal ulcer than in those without peptic ulceration (43,44). However, there frequently is considerable overlap in the number of *H pylori* present in the antrum of subjects with and without duodenal ulcer disease.

Other studies have considered whether host responses to infection may determine whether peptic ulceration will develop. As shown in Table 2, host responses to *H pylori* infection that have been reported to increase the risk of peptic ulceration include altered gastric acid and pepsin production, ectopic gastric mucosa developing in the first part of the duodenum, and inflammation of the duodenal mucosa in response to the production of chemokines by gastric epithelial cells and by immune cells in the inflamed lamina propria of the antrum.

Disruption of gastric acid homeostasis: Before the successful culture of *H pylori* and its identification as a gastric pathogen in humans, peptic ulcer disease was assumed to have a genetic basis in children. Hyperpepsinogenemia appeared to be transmitted in an autosomal dominant fashion in children with recurrent peptic ulcer disease and a strong family history of the same condition. Now, it is clear that the increased levels of serum pepsinogen simply reflect changes in the gastric mucosa occurring as a result of *H pylori* colonization (45,46).

The observed familial nature is a marker of the familial clustering of infection.

In addition to changes in pepsinogen secretion, bacterial colonization of the antral mucosa and the ensuing mucosal inflammation cause changes in the hormones controlling the secretion of acid by parietal cells in the body of the stomach. Both transcription and protein translation of gastrin by G cells and somatostatin by D cells are altered in response to infection and inflammation (47).

Both fasting and postprandial levels of gastrin are elevated in subjects with *H pylori* infection in comparison with age-matched uninfected controls (48). Elevated gastrin levels return towards normal following eradication of the infection. In contrast, somatostatin levels are reduced in *H pylori*-infected individuals. Somatostatin increases to values observed in uninfected persons following anti-helicobacter therapy. The net result of increased gastrin and reduced somatostatin in patients infected with the gastric pathogen is an imbalance in the control of acid secretion that should result in higher levels of acid output from parietal cells. Initial results suggest that this is the case because acid output is higher in patients with *H pylori* infection than in uninfected age-matched and sex-matched controls (49). More importantly, acid output in response to both gastrin-releasing peptide (50) and gastrin (51) is enhanced to a greater extent in subjects with documented duodenal ulcer disease than in *H pylori*-infected patients who have not suffered from peptic ulceration. Other investigators suggest that acid output may be higher in *H pylori*-infected individuals due to a loss of the normal inhibitory effects of antral distension on parietal cell proton output (52).

Gastric metaplasia: *H pylori* colonizes sites outside of the stomach only if there is ectopic gastric mucosa such as in a Meckel's diverticulum in the small bowel (53), Barrett's epithelium in the lower esophagus (54) or gastric rest along the length of the intestinal tract (55). Similarly, *H pylori* is not present in the proximal duodenum in patients with mucosal ulceration unless there are sites of gastric metaplasia.

Initial reports considered the possibility that gastric metaplasia in the first and second parts of the duodenum is an inherited condition predisposing an individual to peptic ulcer disease following infection with *H pylori* during childhood (56). Subsequent studies have shown that this is not the case. Rather, gastric metaplasia occurs in the duodenum in response to *H pylori* infection (57) and regresses following eradication of the bacterial infection from the stomach (58).

Studies in children and adolescents document ectopic gastric mucosa in the duodenum only in children with bacterial colonization of the antrum and not in age-matched controls without infection (59). A prospective study in Irish children showed the presence of gastric mucosa in the proximal duodenum in 86% of those with documented peptic ulcer disease compared with only 20% of 141 children and adolescents with *H pylori*-induced gastritis alone (60). Colonization of the extragastric site may perturb local host defences to an extent sufficient to result in mucosal ulceration. Persisting gastric metaplasia following failure of anti-helicobacter therapy may serve as a marker to identify subjects at increased risk of recurrent peptic ulceration.

Duodenitis: Even more common than ectopic gastric mucosa in the duodenum of subjects with duodenal ulcer disease is the presence of mucosal inflammation (61). Duodenitis is a nearly universal finding in patients with *H pylori* infection and duodenal ulcer disease. It is much less common in subjects with *H pylori* gastritis alone. This difference suggests that the mucosal inflammation may serve as a marker for those at risk of peptic ulceration. It also indicates that inflammation in the duodenum occurring in response to *H pylori* infection in the antrum may be involved in the pathophysiology of disease (62).

It is clear that *H pylori* induces both epithelial cell and immune cell activation of nuclear factor kappa B (63), which, in turn, activates transcription of chemokines including interleukin-8, macrophage inflammatory protein-1alpha, and RANTES (64,65). The chemokines serve as potent chemoattractants that draw polymorphonuclear leukocytes into the gastroduodenal mucosa. A number of other host cytokine responses, including production of interleukin-1, interleukin-6 and tumour necrosis factor-alpha, are also initiated in the *H pylori*-infected gastric epithelium (66,67). Initial studies report that the chemokine and resulting inflammatory responses are more vigorous in infected subjects with duodenal ulcer disease than in those with gastritis alone.

The brisk inflammatory response to *H pylori* colonization of the gastric mucosa also results in the recruitment of T cells (68), plasma cells, mast cells (69), eosinophils (70) and basophils (71) to sites of inflammation. Future studies should determine whether these host inflammatory responses differ between those who have infection complicated by peptic ulceration and those with gastritis alone.

CONCLUSIONS

The final verdict on whether the microbe or the host ultimately determines the outcome of chronic infection remains to be determined. As with many other infections (72,73), a likely possibility is that both the virulence of the infecting organism and the host response to infection work in concert to determine which individuals will remain asymptomatic throughout their lifetime and which will develop complications of infection, including peptic ulceration and malignancies involving either epithelial cells or immune cells in the stomach.

A reproducible model of peptic ulcer disease in an animal model of *H pylori* infection is required to determine the relative contributions of the microbe and the host. Reports of gastric ulcer in the gnotobiotic piglet following challenge with *H pylori* (74) and in the ferret infected with the related gastric pathogen *Helicobacter mustelae* (75) indicate that these are the models that should be employed in future research endeavours. Currently, a model of duodenal ulcer disease complicating *Helicobacter* species infection is lacking. The development of such an animal model should be an urgent and immediate research priority because it ultimately

will provide answers to the questions arising from the issues considered in this review of the virulence properties of *H pylori* and host responses to the gastric infection.

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