Modulation of Pathogenesis by Intestinal Microflora: The Role of *Helicobacter pylori* in Gastroduodenal Ulcer and Gastric Cancer

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Received for publication, July 1, 1998

Helicobacter pylori shows a highly diverse nature in all aspects, from gene profiles to biological features. Interaction between *H. pylori* and host cells is also complex. These characteristics of *H. pylori* may explain some conflicting results regarding the pathogenicity of *H. pylori* in human diseases. Not all strains are pathogenic, as exemplified by Type I and Type II strains. This may possibly lead to the development of a live vaccine using non-toxic strains against toxic strains. To achieve this goal, further study is necessary to elucidate the exact role of *H. pylori* in gastroduodenal diseases.

Key words: *Helicobacter pylori*; gastric microflora; gastritis; peptic ulcer; gastric cancer

HELICOBACTER PYLORI AS A MEMBER OF GASTRIC MICROFLORA

Gastric microflora. The human stomach has been thought to be sterile because of its strongly acidic environment. Microflora can be colonized when acid secretion is ceased or decreased. For example, gastric surgery results in the deprivation of acid-secreting cells together with an alteration of the anatomical structure. Similar environmental change occurs when gastric acid secretion is suppressed by H₂-receptor antagonists or proton pump inhibitors. These environmental changes of the stomach lead to comfortable conditions for infestation by microorganisms (1, 29).

Under normal strong acidity, the infestation of microorganisms is below 10⁵ colony-forming units (CFU)/ml in the stomach. Many of these are opportunistic organisms, though they may be implicated in post-operative sepsis in some cases (63). When gastric acidity decreases, infestation level increases above 10⁵ CFU/ml (1). These organisms are members of gut microflora, and their infestation may induce bacterial overgrowth syndrome or malnutrition status (1).

Spiral microorganisms in the stomach. The presence of spiral bacteria in the stomach was known more than 100 years ago (64). Although several researchers described spiral organisms in the human stomach (13, 22, 65), their presence had been ignored until recent rediscovery by Warren and Marshall in 1983 (39, 72).

Human gastric microflora are summarized in Table 1 together with *H. pylori* (26). Under ordinary conditions, *H. pylori* is a dominant species in the stomach. *H. pylori* is characterized as mucosa-associated bacte-

ria, whereas other bacteria are found in gastric contents. These findings indicate that *H. pylori* is indeed a member of gastric microflora and other bacteria are opportunistic. Thus, *H. pylori* may exert certain actions on the gastric mucosa, whereas other bacteria may exert their actions only when an abnormal condition is induced in the stomach through environmental changes.

Helicobacter pylori. Genus *Helicobacter* includes several gastric and non-gastric species (21). *H. pylori* is a spiral, slightly curved Gram-negative rod with 2 to 7 unipolar flagella. Natural infestation of *H. pylori* is confined to the human and non-human primate stomach. The bacterium possesses urease, and is thereby able to catalyze urea to ammonia and carbon dioxide. The bacterium itself is acid-sensitive, but because of this ammonia production, it can survive under strong acidic conditions (14).

Several factors in addition to ammonia production enable *H. pylori* to colonize in the stomach. Unipolar flagella can expel the bacterium rapidly into the mucous layer, thus avoiding longer exposure in the acidic lumen. Without this mobility the bacterium could not colonize in the stomach (15). Adherence to the epithelium involves binding to Lewis^b blood group antigens (4), which is thought to be one of the reasons for the host preference of *H. pylori*. This adherence phenomenon is also the first step of pathogenesis of *H. pylori* to the human stomach.

Pathogenesis of *H. pylori*. *H. pylori* exerts a variety of pathogenic actions on the gastric mucosa and induces inflammatory response and epithelial cell damage (Fig. 1). Factors involved in these mechanisms include enzymes, toxins, structure substance such as li-

Table 1. Gastric microflora of patients with gastroduodenal diseases with respect to the pH of gastric contents.

	pH < 3 (n = 20)		3 < pH < 6 (n = 18)		pH > 6 (n = 18)	
	Detected cases	Count of organisms	Detected cases	Count of organisms	Detected cases	Count of organisms
Facultative anaerobes	8 (40)	2.4 ± 1.3	14 (78)	6.2 ± 0.6	18 (100)	5.5 ± 1.0
Enterobacteriaceae	0	—	6 (33)	5.9 ± 0.6	5 (28)	4.4 ± 1.3
Streptococci	2 (10)	2.7 ± 0.2	13 (72)	6.2 ± 0.6	16 (89)	5.3 ± 0.9
Micrococcaceae	2 (10)	1.5 ± 0.9	4 (22)	3.0 ± 0.7	4 (22)	4.8 ± 1.7
Yeasts	6 (30)	2.1 ± 1.3	2 (11)	3.5 ± 1.0	3 (17)	4.8 ± 2.0
Bacilli	2 (10)	3.7 ± 1.1	1 (6)	4.0	0	—
Lactobacilli	2 (10)	2.8 ± 0.5	8 (44)	4.5 ± 0.5	9 (50)	3.6 ± 0.7
Obligate anaerobes	0	—	9 (50)	4.8 ± 1.2	10 (56)	4.0 ± 1.4
Bifidobacteria	0	—	4 (22)	4.6 ± 1.0	5 (28)	3.1 ± 0.9
Eubacteria	0	—	1 (6)	2.6	1 (6)	3.2
Bacteroidaceae	0	—	5 (28)	3.7 ± 0.7	2 (11)	4.3 ± 0.9
Peptococcaceae	0	—	3 (17)	6.1 ± 0.5	4 (22)	4.9 ± 1.1
Clostridia	0	—	0	—	1 (6)	3.5
<i>Helicobacter pylori</i>	12 (60)	5.1 ± 1.2	11 (61)	6.1 ± 1.0	14 (78)	5.9 ± 1.4
Total	14 (70)	5.3 ± 1.3	13 (72)	6.6 ± 0.6	18 (100)	6.1 ± 1.3

Results are shown by number of detected cases with % in parentheses, and counts of organisms are mean ± SD (log/ml) among detected cases.

Fig. 1. An overview of the pathogenesis of *H. pylori*. LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; MPO, myeloperoxidase.

polysaccharide (LPS) and heat-shock proteins (Hsp).

Enzymes secreted by the bacterium include urease, glycosulfatase, phospholipase and others. Ammonia produced by urease directly damages gastric epithelial cells (47). Glycosulfatase may disturb the mucous layer

of the stomach and decrease the protective role of the mucous layer against acid (43). Phospholipase can cleavage membrane phospholipids and damage the membrane structure of gastric epithelial cells (11).

H. pylori produces toxins. More than 50% of the

strains have toxin inducing vacuolization of epithelial cells (20). Strains which produce this toxin (VacA) are designated as Type I. In general, Type I strains are associated with more severe pathogenicity on the gastric mucosa than Type II strains, which do not have VacA (9, 38). Cytotoxin-associated gene A, *cagA*, is found in almost all VacA-positive strains. Later, the *cag* pathogenicity island was identified and its role in virulence was assessed (6). Mutants of *cag* showed a reduction of interleukin-8 secretion from gastric epithelial cells (61). Thus, *cag* is involved in *H. pylori*-host cell interaction and associated with the chronic inflammatory reaction of the gastric mucosa induced by *H. pylori*.

Hsps or chaperonins are highly immunogenic. Because of this property, some role of Hsps is speculated in the immune system (49), although there is no association between Hsps and disease. Experimentally, atrophic gastritis is induced in the LPS-sensitive strain but not in the LPS-insensitive strains of mice (59). The induction of mononuclear cells to LPS-sensitive mice indicated that LPS induced an inflammatory response through cellular immune response (59).

The toxicity of the LPS of *H. pylori* itself is lower compared with that of other bacteria (45). This finding is ascertained by the characteristic of Lipid A, a component of LPS, of *H. pylori*. The induction of interleukin-8 and tumor necrosis factor- α from fibroblasts is lower, while the induction of interleukin-6 and natural killer cell activity is comparable to that of *Escherichia coli*-Lipid A (28). These results suggest that the biological activity of *H. pylori*-LPS is relatively low, while its immunogenicity is maintained.

H. pylori infection increases pro-inflammatory cytokines (10). Among them, interleukin-8, which is secreted by gastric epithelial cells, is an important host mediator for neutrophil migration and activation (10). Neutrophil activation and the production of reactive oxygen metabolites cause mucosal injury (46, 66). The active components of *H. pylori* mentioned earlier play a role in these inflammatory responses and interactions between *H. pylori* and host cells. Also, the degree of these changes and mucosal injury is more severe in Type I than Type II strains.

These characteristics of *H. pylori* suggest that this bacterium is pathogenic. However, *H. pylori* may play a protective role under certain conditions (35). Therefore, whether *H. pylori* is commensal or pathogenic in the stomach is not a simple issue.

H. pylori-induced gastritis. Pathogenicity of *H. pylori* eventually induces gastritis. Indeed, *H. pylori* is a major cause of human gastritis. Originally, *H. pylori*

was found from the gastric mucosa with chronic inflammation (39, 72). A challenge of *H. pylori* (i.e., ingestion of *H. pylori*) by previously *H. pylori*-negative volunteers resulted in histological and clinical gastritis (40, 44). *H. pylori* is detected in more than 85% of the patients with chronic inflammation of the gastric mucosa including polymorphonuclear and mononuclear cells. Eradication of *H. pylori* results in the regression of these cell infiltrations (56).

When infestation of *H. pylori* persists, a significant proportion of non-atrophic gastritis progresses to atrophic gastritis (33); the rate of transition from non-atrophic to atrophic gastritis was estimated to be 2.1 to 2.6% per year for *H. pylori*-positive patients (71). The severity and density of *H. pylori* infestation vary depending on different diseases. Thus, gastritis is generally restricted in the antral region in patients with duodenal ulcer, whereas gastritis in gastric ulcer and cancer patients is distributed more widely in the body of the stomach (37).

H. PYLORI AND PEPTIC ULCER

H. pylori in peptic ulcer. *H. pylori* has been found more frequently in patients with gastroduodenal ulcer than healthy subjects. The age of *H. pylori* infection is thought to be important in the development of gastric and duodenal ulcers. Namely, an earlier establishment of *H. pylori* infection is a risk factor for gastric ulcer but not duodenal ulcer (2). However, an Indian experience, i.e., high prevalence rate of *H. pylori* in younger age groups and duodenal ulcer, is not in accord with this theory.

The evidence of a relationship between *H. pylori* and peptic ulcer has emerged from clinical experience. The recurrence rate of peptic ulcer is quite high, whereas the eradication of *H. pylori* completely prevents ulcer recurrence (25). Although many studies including ours (Fig. 2) do not concur with the complete prevention of ulcer recurrence (24), there is no doubt that *H. pylori* is a risk factor for ulcerogenesis.

Mechanism of ulcer formation by H. pylori. Factors involved in mucosal injury and the induction of gastritis are likely to be contributory to ulcerogenesis as well. For example, ammonia produced by *H. pylori* induces gastric ulcer experimentally (47). Gastric metaplasia harboring *H. pylori* occur in the duodenal bulb (75), which may directly damage duodenal mucosa. However, these findings have offered little evidence regarding the exact mechanism of *H. pylori*-related ulcer formation in humans.

H. pylori colonizes mostly in the antrum of patients

Fig. 2. Cumulative non-recurrence rate of gastric ulcer with respect to eradication of *H. pylori*.

with duodenal ulcers (37). Local acid secretion and colonization of *H. pylori* are closely associated (12). *H. pylori* infection blocks the physiological inhibitory effect of gastric acid secretion induced by antral distension, which results in an increased duodenal acid load (51). This abnormal response is restored by the eradication of *H. pylori* (51). This acid hypersecretion and mucosal damage caused by *H. pylori* are critically important factors contributing to ulcer formation.

Management of gastroduodenal diseases. Although there are unsolved questions regarding the role of *H. pylori* in peptic ulcer, the eradication of *H. pylori* is likely to be a reasonable option for the management of peptic ulcer. Thus, consensus guidelines have been reported at conferences worldwide (16, 36, 48).

These conferences discussed the role of *H. pylori* and implications of its eradication in gastroduodenal diseases. Although there are small differences among the 3 guidelines, they unanimously recommend that ulcer patients with *H. pylori* infection receive eradication therapy whether the ulcer is active or in remission.

In recommendable regimens, above 80%, preferably over 90% eradication is necessary. At present, the combination of a proton pump inhibitor and 2 antibiotics have offered a satisfactory outcome (58).

H. PYLORI IN GASTRIC CANCER

Association of *H. pylori* and gastric cancer. The epidemiological findings of gastric cancer coincided with those of *H. pylori*. The carrying rate of *H. pylori* is high in Japanese (26) and in people with a high risk of gastric cancer (8). Epidemiological studies have confirmed the positive association of *H. pylori* infection and gastric carcinogenesis prospectively (19, 50, 54). According to Parsonnet, 1% of *H. pylori*-infected

persons will eventually develop gastric cancer (52).

Additional findings. With regard to experimental findings, because of the lack of an adequate animal model for *H. pylori* infestation, there is little evidence of the carcinogenic effect of *H. pylori*. In a rat MNNG model, the loading of *H. pylori* did not promote gastric carcinogenesis (30). A series of experimental studies were also inconclusive (67, 68).

The recent introduction of Mongolian gerbils to *H. pylori* experiments has given additional findings to this issue. Promoting and co-initiating effects were observed by *H. pylori* infection in MNU-induced gastric carcinogenesis in these animals (41). More interestingly, a 62-week period of *H. pylori* infection resulted in the formation of adenocarcinomas in 37% of the infected animals (73). These results indicate that *H. pylori* has both initiating and promoting activity toward gastric carcinogenesis. Further study is needed to confirm these findings.

In a non-blinded study, the eradication of *H. pylori* resulted in the resolution of inflammation and intestinal metaplasia of the gastric mucosa and inhibited the development of new early gastric cancer (70). This finding should be examined in a blinded manner. Furthermore, the fundamental question, "What is early gastric cancer?" (17) must be settled before reaching any conclusion regarding this finding.

The role of *H. pylori* in gastric carcinogenesis. Based on epidemiological findings, *H. pylori* was defined as a "definite biological carcinogen" by WHO/IARC in 1994 (27). *H. pylori* induces atrophic gastritis (33), and this effect is greater in *cagA*-positive than *cagA*-negative strains (32). Thus, the role of *H. pylori* in gastric carcinogenesis is thought to be indirect, i.e., the induction of atrophic gastritis as a first step in the gastritis-metaplasia-carcinoma sequence (7). A recent study on telomerase indicated that *H. pylori* was involved in metaplastic change (34), which may fill in the missing link for the role of *H. pylori* in the gastritis-metaplasia-carcinogenesis sequence.

The direct involvement of *H. pylori* is also indicated by several studies including the latest reports, which showed the initiating and promoting activity of *H. pylori* in gastric carcinogenesis in Mongolian gerbils (41, 73). *H. pylori* causes neutrophil infiltration, and activated neutrophils induce DNA damage. Indeed, a parameter of DNA damage accumulated when *H. pylori* infected the stomach (18). A decrease in the level of vitamin C due to *H. pylori* infection may accelerate this process (57). Increased cell proliferation without a corresponding increase in apoptosis during the infection

of *cagA*⁺ strains may be a risk factor for the development of cancer from unlimited cell proliferation (55). Genetic abnormalities involving DCC and APC genes were described during transition from chronic gastritis to MALToma (5). Furthermore, ammonia, a product of *H. pylori*, promoted experimental gastric carcinogenesis (69).

Yet, several issues have been left to be solved regarding the carcinogenic role of *H. pylori*. The inverse relationship in epidemiological findings between gastric cancer and duodenal ulcer conflicts with the causal implication of *H. pylori* in gastric carcinogenesis. Further, *H. pylori* is not the sole factor for causing atrophic gastritis (60). Unlike MALToma (5), DCC, APC and other genes did not have any association with *H. pylori* infection (74). Unlike studies from Western countries (19, 50, 54), epidemiological studies from countries with a high incidence of gastric cancer do not support the involvement of *H. pylori* in gastric carcinogenesis (23, 31). On the other hand, gastric carcinogenesis in younger age groups was likely to be affected by *H. pylori* infection (3, 23). The association of *cagA*-positive strains and gastric cancer observed in Western countries (53) was not the case in Japan (42, 62).

Thus, the role of *H. pylori* in gastric carcinogenesis is likely to be different between countries with high and low incidences of gastric cancer. The action of *H. pylori* is probably dependent on stages of the gastritis-metaplasia-carcinoma sequence. Also, host factors as well as other environmental factors may be important in the action of *H. pylori* in carcinogenesis.

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