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III. Virulence and pathogenicity of *Helicobacter pylori*

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Abstract *H. pylori* is a highly virulent organism as evidenced by its low infective dose and widespread high prevalence in human populations. Its virulence is achieved through its ability to survive in a moist environment and its massive urease production which allows it to survive in the acidic gastric juice long enough to colonize the gastric mucus.

Gastric colonization is facilitated by cell wall associated lectins which permit the bacterium to bind to gastric mucus and the gastric epithelial cell. Once in this location, *H. pylori* produces several enzymes which may harm the gastric epithelium, particularly urease (through ammonia generation) and phospholipases A and C. *H. pylori* also weakens the gastric mucous layer by digesting its glycoproteins and lipids, making the mucus less hydrophobic and more water soluble.

Helicobacter pylori attracts phagocytic cells, inducing both acute and chronic inflammation as well as an antibody response. Persistence of *H. pylori* in the mucosa may be enhanced by its cytotoxin and catalase production, by which it survives after phagocytosis by neutrophils.

Key words: animal models, *Campylobacter*, catalase, cytotoxin, gastritis, *Helicobacter pylori*, pathogenicity, peptic ulcer, urease, virulence.

INTRODUCTION

The success of a pathogen depends on both its virulence and its pathogenicity. Virulence is the ability to infect a host, whereas pathogenicity is the ability to cause a disease in the host.

For enteric bacteria, sufficient numbers of viable organisms must survive the gastric acid barrier and colonize the enteric fluid or mucous layer. Examples of important virulence factors are attachment mechanisms and motility in the intestinal mucous layer. Once the organism is established in the gut, pathogenic effects on the host may be produced by one or several means; examples are physical effects, elaboration of enzymes or toxins, and competition with the host for nutrients.

Spiral shape and flagellar motility in mucus

Bacteria which colonize mucus are often spiral in shape—examples are spirochaetes, which colonize the genital tract, or Campylobacters, such as *Campylobacter jejuni*, which colonize the gut of animals and humans. *Helicobacter pylori* is spiral, with a wavelength of approximately 2 μm and usually 1.5 λ per organism. Hazell *et al.* showed that this spiral shape is well adapted to motility in the viscous gastric mucous layer.[1] In a study using increasing concentrations of methylcellulose, *H. pylori* was able to travel much greater distances than flagellated non-spiral organisms such as *E. coli*. After passage on artificial media, *H. pylori* isolates may lose their motility and form less virulent 'granular' colonies.[2]

Binding to mucus and epithelial cells

Lectins are molecules (usually proteins) which bind selectively with carbohydrate moieties on mammalian cells. The most commonly used lectins are derived from plants. Many bacteria, including *H. pylori*, have cell wall associated lectins which allow them to bind selectively to mucus and epithelial cells. Targets for *H. pylori* lectins exist in the gastric mucus as glycoproteins and glycolipids. *Helicobacter pylori* appears to bind to all of these, including sulfated (acid) mucins, L-fucose, D-galactose and sialic acids.

Emody *et al.* showed that *H. pylori* lectins attach to red cells from various animal species.[3] Such attachment can be blocked by mono-sialogangliosides. Similar investigations by Evans *et al.* indicated that *H. pylori* attached to *N*-acetylneuraminyllactose by a fibrillar protein structure.[4] Thus, attachment of *H. pylori* to epithelial cells is similar to that found in enteropathogenic *E. coli* infection, where a fibrillar adhesin attaches to carbohydrate moieties on the intestinal cells, resulting in pedestal formation where the bacterium adheres.[5]

Presently, the most likely target for *H. pylori* is a ganglioside, Gm3. Gangliosides consist of a membrane-bound sphingolipid attached to a carbohydrate end-group. They vary in structure depending on species and cell type, and may disappear when malignant transformation of cells occurs. They often act as bacterial targets — for example,

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cholera toxin attaches to ganglioside Gm1. Ganglioside Gm3 has both a sialic acid group and a lactose moiety.

Recently, Lingwood *et al.* described a glycerolipid which attracts *H. pylori*.[6] In their experiments, glycerolipid was isolated from human and pig stomachs and blotted onto a membrane. After incubation, *H. pylori* organisms were seen to selectively attach to areas of the membrane upon which the glycerolipid was deposited. Charged glycerolipids are present in many different cell types and may explain why some types of *H. pylori* attachment cannot always be blocked with carbohydrate lectins.

Tight attachment to cells

Helicobacter pylori attaches tightly to the epithelial cell, and a characteristic structure called an 'attachment pedestal' forms.[5] Tight attachment of *H. pylori* to epithelial cells appears to cause localized cell damage characterized by effacement of microvilli and disruption of the cytoskeletal elements of the cell. The cytoskeleton of the epithelial cells appears quite abnormal when *H. pylori* is attached.[5] Smoot *et al.* found that actin polymerization occurs below the sites of attachment pedestals for *H. pylori*, an ultrastructural abnormality identical to that seen in enteropathogenic *E. coli* infections.[7]

Elaboration of enzymes

Urease and ammonia

The most obvious of the *H. pylori* enzymes is urease, known to exist in the stomach of humans for more than 40 years but previously thought to be of mucosal origin.[8] *Helicobacter pylori* urease is highly active between the pH of 5 and 8, has a Km of around 1 mmol/L urea (a physiologic concentration) and appears to be an extremely important component of organisms which colonize the mammalian stomach.

Since all gastric spiral bacteria so far isolated have been urease positive, urease and ammonia production may be important virulence factors for *H. pylori*. One of the major functions of gastric acid is to sterilize food before it enters the small intestine. Even *Salmonella* cannot survive more than 30 min in gastric juice at pH 2.0,[9,10] but *H. pylori* survives in gastric acid by metabolizing the urea present. Even 0.1 mmol/L urea will substantially protect *H. pylori* from acid attack so that only a 2–3 log reduction in bacteria occurs after 30 min at pH < 2.0.

Ammonia is toxic to mammalian cells because it combines with alphaketoglutarate to form glutamine, thus depleting the TCA cycle of an essential intermediate substrate.[11] Ammonia generated by urea and urease has been shown to cause epithelial cell damage in rats. This effect may be mediated by neutrophil-generated oxygen radicals and HOCl because it is inhibited by anti-neutrophil serum. The effect is also inhibited by taurine and methionine, agents which scavenge monochloramine (NH_2Cl), the toxic metabolite of hypochlorous acid.[12] Recently, Xu *et al.* produced vacuolation of epithelial cells *in vitro* by incubating them with urease and urea. Thus some of the obvious cytopathic effects of *H. pylori* may be secondary to its urease.[13]

The association of *H. pylori* with hypochlorhydria has been noted by many authors and is thought to be secondary to parietal cell failure. Presumably, *H. pylori* produces a toxin which prevents the parietal cell from secreting acid. Ammonia itself may be such a 'toxin'. Ammonium ions compete with hydrogen ion for the hydrogen potassium ATPase of the parietal cell membrane so that, in the presence of ammonia, ammonium ions rather than hydrogen ions are excreted from the parietal cells. In addition, Cave *et al.* showed that a soluble protein from *H. pylori* is able to prevent acid secretion from cultured parietal cells.[14] Since patients with duodenal ulcer produce acid in at least normal amounts, the parietal cell toxin may be of relevance only in the acute *H. pylori* infection where heavy colonization of the corpus mucosa occurs.

Catalase

Catalase may be an important protective enzyme for *H. pylori*. *H. pylori* produces very large amounts of catalase, as do other members of the *Helicobacter* family. Hazell suggests that release of catalase into the extracellular milieu may protect *H. pylori* from toxic long chain fatty acids (e.g. arachidonic acid) and their metabolites (lipid peroxides) produced by lysis of neutrophils.[15] Similarly, catalase may protect *H. pylori* from phagocytosis. Normally, after ingestion of a bacterium, neutrophils perform an 'oxidative burst', in which NADP+ is converted to NADPH and superoxide (O_2^-) is generated. Superoxide is converted by superoxide dismutase to hydrogen peroxide (H_2O_2). H_2O_2 is then converted by myeloperoxidase, in the presence of halides, to HOCl and other bactericidal products.[12,16] Single oxygen (^1O) and hydroxyl ($OH^\cdot$) radicals are also formed, resulting in peroxidation of bacterial lipids and denaturation of proteins.

When catalase is ingested with or as part of bacteria, all H_2O_2 is immediately broken down to H_2O and O_2, thus preventing the production of these toxic radicals. It can be shown that bacterial killing is markedly impaired in the presence of catalase.[17] This may be why mucosal infection with *H. pylori* is difficult to eradicate.

Proteases and digestion of the gastric mucus

The normal gastric mucus is susceptible to acid peptic digestion because it consists of a protein backbone with numerous attached carbohydrate moieties. Logically, such digestion can only take place at the luminal surface of the mucus layer. In patients with gastric ulcer, analysis of mucus reveals a large component of degraded mucin, expressed as a second peak after passage through a gel column.[18] Sarosiak *et al.* showed that incubation of mucus with *H. pylori* reproduces this second peak.[19] This may be an important phenomenon, since the digestion occurs within the mucus layer rather than from without.

Phospholipases A2 and C

The epithelial cell membrane consists of a phospholipid bilayer. Phospholipids are similar to triglycerides except that one of the terminal fatty acids is replaced by a phosphate group. Phospholipase A2 of *H. pylori* removes a long-chain fatty acid group from the second carbon.[20] Phospholipase C removes the phosphate group from the third carbon of the phospholipids. The resultant compounds, diacyl glyceride and particularly lysolecithin, are incapable of forming the normal phospholipid bilayers and may form micellar structures instead, potentially affecting the integrity of the epithelial cell membrane.

Phospholipase A2 is also an activator of the inflammatory response. It attacks membrane phospholipids to liberate arachidonic acid which may then be converted to leukotriene, prostaglandin or thromboxane. These compounds are known to cause mucus release, chemotaxis of inflammatory cells and altered membrane permeability.[21]

Another effect of phospholipases is the potential ability to impair the normal protective effect of the gastric mucous layer. The ability of the mucous layer to repel water is dependent on its phospholipid content and is known to be decreased in the mucus of ulcer patients. Goggin *et al.* showed that *H. pylori*-infected mucus is less hydrophobic and that artificial phospholipid layers also lose their hydrophobicity when exposed to phospholipases.[22]

Toxins

Helicobacter pylori vacuolating cytotoxin can be demonstrated *in vitro* by observing its effect on various cultured cell lines (e.g. vero, HeLa, Intestinal 407 and Chinese Hamster Ovary (CHO)) cells. The toxin has been characterized by Leunk *et al.*[23] It is heat labile at 70°C and is susceptible to trypsin digestion, indicating that it is a protein. The molecular weight of the toxin is approximately 80 kDa. The vacuolating cytotoxin may be quantitated by applying serial dilutions of *H. pylori* extract to *in vitro* cell cultures. Using the method described by Leunk *et al.*, up to 60% of *H. pylori* isolates have detectable cytotoxin.[23]

Eaton *et al.* challenged gnotobiotic piglets with *H. pylori* strains with varying motility and cytotoxin production.[2] Their data showed a tendency for more motile strains to produce a higher infection rate. The most virulent isolate of *H. pylori* had a high cytotoxin production and was highly motile. Less motile isolates regained motility after passage through the pigs. These authors believe that motility is a more important virulence factor than the presence of vacuolating cytotoxin.

The vacuolating cytotoxin of *H. pylori* may be an important pathogenic factor, according to Figura *et al.*[24] They found that, in persons with a duodenal ulcer, *H. pylori* was usually cytotoxic (66%) whereas persons with non-ulcer dyspepsia and gastritis had a lower prevalence of cytotoxic *H. pylori* isolates (30%). Cover *et al.* found a similar association with cytotoxin and duodenal ulcer; in their study 100% of patients with duodenal ulcer had a 'toxin band' on immunoblot, compared with 60% of patients with 'non-ulcerogenic' *H. pylori* infections.[25]

Causation of inflammation

Very little is known about mucosal immunity in the stomach, so this discussion relates mainly to possible mechanisms whereby *H. pylori* could incite a local reaction and thus impair the acid protection function of the gastric epithelium.

The chronic gastritis associated with *H. pylori* has a striking histological appearance, particularly in gastric ulcer patients. Whereas normal gastric mucosa has very few mononuclear cells present, most of the lamina propria may be replaced by such cells in infected patients. The severity of epithelial cell damage is proportional to the number of *H. pylori* organisms with tight attachment to the epithelium.[26] Under high power light microscopy, small micro-abscesses are often seen, in which *H. pylori* organisms disrupt an epithelial cell and multiply in direct contact with the underlying basement membrane. In this circumstance bacterial antigens may gain access to the lamina propria and stimulate the observed inflammatory response.

The hallmark of *H. pylori* colonization is the presence of neutrophils in the gastric epithelium, the number of which is inversely proportional to the amount of tightly attached *H. pylori*.[26] One chemotactic factor responsible for attracting neutrophils *in vitro* is a low molecular weight (<3000) peptide.[27]

Once phagocytic cells come into contact with *H. pylori* antigens, the oxidative burst (already described) occurs with subsequent release of oxygen and hydroxyl radicals toxic to *H. pylori* and the mucosal cells. Monocytes exposed *in vitro* to *H. pylori* lipopolysaccharide have been shown to exhibit type 2 transplantation antigens and interleukin-2 receptors (a sign of 'activation'), and also to release superoxide, interleukin-1, prostaglandin E2 and tumour necrosis factor. Interleukin-1 and tumour necrosis factor decrease neutrophil chemotaxis (prevent the cells from leaving the area) and trigger further production of superoxide. In parallel with this, IgG produced by intraepithelial plasma cells may combine with *H. pylori* soluble antigens to activate the complement pathway. Activated complement can cause further neutrophil chemotaxis (C5a) and cell lysis. The sudden resolution of chronic gastritis following eradication of *H. pylori* is compelling evidence that the bacterium is responsible for these changes.[28]

CONCLUSION

Helicobacter pylori is a highly virulent organism as evidenced by its widespread high prevalence in human populations. Its virulence is achieved through its massive urease production, which allows it to survive in the acidic gastric juice and colonize the gastric mucus. Colonization is facilitated by membrane-bound lectins which permit the bacterium to bind to the gastric epithelial cell. Once in this location, *H. pylori* produces several other enzymes which may harm the gastric epithelium, particularly urease (through ammonia generation) and phospholipases A and C. *Helicobacter pylori* also weakens the gastric mucous layer by digesting its glycoproteins and lipids, making it less hydrophobic and more water soluble. *Helicobacter pylori* attracts phagocytic cells, inducing both acute and chronic inflammation as well as an antibody response. Persistence of *H. pylori* in the mucosa may be enhanced by its cytotoxin production and catalase enzyme which allows it to survive after phagocytosis by neutrophils.

REFERENCES

1. Hazell S. L., Lee A., Brady L. & Hennessy W. *Campylobacter pyloridis* and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. *J. Infect. Dis.* 1986; **153**: 658–63.

2. Eaton K. A., Morgan D. R. & Krakowka S. *Campylobacter pylori* virulence factors in gnotobiotic piglets. *Infect. Immun.* 1989; **57**: 1119–25.
3. Emody L., Carlsson A., Ljungh A. & Wadstrom T. Mannose-resistant haemagglutination by *Campylobacter pylori*. *Scand J. Infect Dis.* 1988; **20**: 353–4.
4. Evans D. G., Evans D. J. Jr, Moulds J. J. & Graham D. Y. N-acetylneuraminyllactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor antigen. *Infect. Immun.* 1988; **56**: 2896–906.
5. Goodwin C. S., Armstrong J. A. & Marshall B. J. *Campylobacter pyloridis*, gastritis, and peptic ulceration. *J. Clin. Pathol.* 1986; **39**: 353–65.
6. Lingwood C. A., Law H., Pellizzari A., Sherman P. & Drumm B. Gastric glycerolipid as a receptor for *Campylobacter pylori*. *Lancet* 1989; **2**: 238–41.
7. Smoot D. T., Mobley H. L. T., Gilliam T., Phelps P. & Resau J. H. Pedestal formation of *Helicobacter pylori* with gastric epithelial cells *in vitro* may require actin polymerization. *Gastroenterology* 1989; **5**: A127 (abstr).
8. Fitzgerald O. & Murphy P. Studies on the physiological chemistry and clinical significance of urease and urea with special reference to the human stomach. *Irish J. Med. Sci.* 1950; **292**: 97–159.
9. Giannela R. A., Broitman S. A. & Zamcheck N. Gastric acid barrier to ingested microorganisms in man: studies *in vivo* and *in vitro*. *Gut* 1972; **13**: 251–6.
10. Marshall B. J., Barrett L., Prakash C., McCallum R. W. & Guerrant R. L. Urea protects *C. pylori* from the bactericidal effect of acid. *Gastroenterology*, 1990; **99**: 697–702.
11. Lehninger A. L. *Biochemistry*. Worth Publishers Inc., NY. 1978: 583.
12. Murakami M., Yoo J. K., Teramura S., Yamamoto K. & Kita T. Protection of rat gastric mucosa by scavengers of hypochlorous acid: role of ammonia and neutrophils. *Gastroenterology* 1989; **5**: A93 (abstr).
13. Xu J. K., Goodwin C. S., Cooper M. & Robinson J. Intracellular vacuolization caused by the urease of *Helicobacter pylori*. *J. Infect. Dis.* 1990; **161**: 1302–4.
14. Cave D. R. & Vargas M. Effect of a *Campylobacter pylori* protein on acid secretion by parietal cells. *Lancet* 1989; **ii**: 187–9.
15. Hazell S. L. & Graham D. Y. Unsaturated fatty acids and viability of *Helicobacter* (*Campylobacter*) *pylori*. *J. Clin. Microbiol.* 1990; **28**: 1060–1.
16. Barbour B. M. Oxygen dependent microbial killing by phagocytes. *N. Engl. J. Med.* 1978; **298**: 659–68.
17. Roos D., Weening R. S., Wyss S. R. & Aebi H. A. Protection of human neutrophils by endogenous catalase. *J. Clin. Invest.* 1980; **65**: 1515–22.
18. Slomiany B. L., Bilski J., Sarosiek J. *et al. Campylobacter pyloridis* degrades mucin and undermines gastric mucosal integrity. *Biochem. Biophys. Res. Commun.* 1987; **144**: 307–14.
19. Sarosiek J., Slomiany A. & Slomiany B. L. Evidence for weakening of gastric mucus integrity by *Campylobacter pylori*. *Scand. J. Gastroenterol.* 1988; **23**: 585–90.
20. Slomiany B. L., Nishikawa H., Piotrowski J., Okazaki K. & Slomiany A. Lipolytic activity of *Campylobacter pylori*: effect of sofalcone. *Digestion* 1989; **43**: 33–40.
21. Ward P. A. Mechanisms of tissue injury: Inflammation. Chapter in Bellanti J. A. ed., *Immunology* III. J. B. Saunders Co. Philadelphia 1985: pp. 210–3.
22. Goggin P. M., Marrero J. M., Jazrawi R. P., Corbishley C. M., Yu C. M. & Northfield T. C. Effect of *Helicobacter pylori* infection on gastric mucosal hydrophobicity in man. *Gastroenterology* 1989; **5**: A49 (abstr).
23. Leunk R. D., Johnson P. T., David B. C., Kraft W. G. & Morgan D. R. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. *J. Med. Microbiol.* 1988; **26**: 93–9.
24. Figura N., Guglielmetti P., Rossolini A. *et al.* Cytotoxin production by *Campylobacter pylori* strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. *J. Clin. Microbiol.* 1989; **27**: 225–6.
25. Cover T. L., Dooley C. P. & Blaser M. J. Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. *Infect. Immun.* 1990; **58**: 603–10.
26. Hessey S. J., Spencer J., Wyatt J. I. *et al.* Bacterial adhesion and disease activity in *Helicobacter* associated chronic gastritis. *Gut* 1990; **31**: 134–8.
27. Craig P. M., Karnes W. E., Territo M. C. & Walsh J. H. *Helicobacter pylori* secretes a chemotactic factor for monocytes and neutrophils. *Gastroenterology* 1989; **5**: A33 (abstr).
28. Marshall B. J., Armstrong J. A., Francis G. J., Nokes N. T. & Wee S. H. Antibacterial action of bismuth in relation to *Campylobacter pyloridis* colonization and gastritis. *Digestion* 1987; **37**: 16–30.