The pathogenesis of *Helicobacter pylori* infection

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**SUMMARY**

It is well established that the presence of *Helicobacter pylori* in the gastric mucosa is associated with chronic active gastritis and is implicated in more severe gastric diseases, including chronic atrophic gastritis (a precursor of gastric carcinomas), peptic ulceration and mucosa-associated lymphoid tissue lymphomas. On the other hand, it is well recognized that only a minority of infected individuals develop severe inflammation leading to peptic ulcer or gastric cancer. In an effort to evaluate the factors that could determine the clinical outcome of infection, investigators focused on virulence factors of the organism, but substantial data failed to support this model. In the present study we tried to overview the mechanisms involved in the pathogenesis of *Helicobacter pylori* infection in humans, emphasizing the factors which are likely to be more crucial in disease progression to peptic ulcer disease, MALT lymphomas and gastric cancer. It seems likely that other host-derived and environmental factors are more significant in determining clinical outcome but additional studies are needed in order to clarify the underlying mechanisms involved in the clinical outcome of infection.

**Key words:** Helicobacter pylori, gastritis, gastric cancer, MALT lymphoma, peptic ulcer

**INTRODUCTION**

*Helicobacter pylori* (Hp) is a micro-aerophilic, Gram-negative, slow-growing, spiral-shaped and flagellated organism. Its most characteristic enzyme is a potent multishubunit urease that is crucial for its survival in acidic pH and for its successful colonization of the gastric environment, an area that few other microbes can colonize. Hp infection is probably the most common chronic bacterial infection in humans, present in almost half the world population. The presence of the bacterium in the gastric mucosa is associated with chronic active gastritis and is implicated in more severe gastric diseases, including chronic atrophic gastritis (a precursor of gastric carcinomas), peptic ulceration and mucosa associated lymphoid tissue lymphomas.

Because of its importance as a human pathogen investigators have sequenced the complete genome of two representative Hp strains (26695 and J99) by the whole-genome random sequencing method. Comparing Hp genes with genes of known function in other bacteria gave immediate insights into Hp metabolism, structure, adaptive mechanisms and virulence. In addition, comparison of the genomic sequence of the two independent clinical isolates has shown that they are highly conserved, with only 7% of the proteins being strain specific.

The pathogenesis of Hp-associated gastroduodenal disease remains poorly understood. It is clear that only a minority of infected individuals develop severe inflammation leading to peptic ulcer or gastric cancer. What are the factors which determine whether an infected individual will develop severe disease?

1. *Helicobacter pylori* - related factors

Virulence factors of Hp may be divided into colonization factors, factors that allow it to evade the host defences, and factors that are responsible for tissue injury.

1.a. Colonization factors

Colonization factors are attributes of an organism that allow it to establish its presence and to persist despite the host’s attempts to rid himself of infection.
Flagella and Motility

Hp has been shown to require flagella for infection of the stomach. Flagella allow the bacterium to swim across the viscous gastric mucus and reach the more neutral pH below the mucus. To analyze whether flagella themselves or motility is needed by these pathogens, investigators constructed flagellated nonmotile mutants. Their results support a model in which motility is used for the initial colonization of the stomach and also to attain full infection levels.4

Urease system

Hp synthesizes urease constitutively. Since urease hydrolyzes urea to form ammonia and carbon dioxide, and ammonia can absorb acid to form ammonium, it is natural to suspect that this dedication to make urease has a relationship to survival and growth in the acidic environment of the human stomach. This suspicion has been confirmed in animal models but it is not certain that the requirement for urease is for colonization as well as for infection.3

There are data showing that the organisms do buffer the periplasm that lies between their inner and outer membrane, in acidic pH, using their intrabacterial urease activity.6 In contrast to surface or free urease, measurement of intrabacterial urease activity at different pH values, shows low urease activity at neutral pH, rapid increase between pH 6,0 and 5,0 and steady activity down to a pH of 2,5 but still present at pH 2,0.7 Expression of the Hp ureI gene is required for acidic pH activation of cytoplasmic urease.8

Adhesins

Hp selectively binds to gastric epithelial cells and its colonization of the digestive tract is limited to areas lined by gastric type epithelial cells. On adhesion, tyrosine phosphorylation and cytoskeletal rearrangement occurs, leading to a remodelling of the apical surface of the epithelial cells.9 Several epithelial structures have been implicated in adhesion, including lipids, gangliosides and sulfated carbohydrates, but, to date, the adhesins on the bacterial surface that bind to the epithelium are poorly understood.

As of yet, no adhesions have been confirmed as being important for in vivo survival of Hp. With the sequence of the H. pylori genome in hand, it should be possible to more easily determine the role of specific genes in virulence. Genes of immediate interest are the OMPs, which may undergo phase and antigenic variation and may represent adhesions.10 Adhesion is necessary for the initiation of the inflammatory cascade. In particular adhesion is a prerequisite for IL-8 secretion by gastric epithelial cells.11 Adherence also promotes the development of more severe disease. BabA adhesin, binds the Lewis b blood group antigen on the gastric epithelium, and is associated with duodenal ulcer, distal gastric cancer and more severe gastritis.12

Other factors which may also participate in Hp adhesion are AlpA and AlpB. Both of these are required for adhesion to human gastric tissue sections.13 BabA and Alp proteins are members of the large family of related outer membrane proteins (Hop proteins). These proteins are not present in all strains of Hp and thus may represent means by which the pathogen gains control of the host response.

A recently described adhesin is a sialic acid-binding adhesin (SabA). The ability of many Hp strains to adhere to sialylated glycoconjugates expressed during chronic inflammation might contribute to virulence and the extraordinary chronicity of Hp infection.14

Heat shock proteins (Hsp)

Hp expresses two heat shock proteins, A and B. They are highly antigenic. The clinical outcomes of H. pylori infection are not related to HspA antigenicity or to sequence variation.15 Recent data suggest that a common epitope is present in human hsp60 and its bacterial homologue hspB.16 Thus infection with Hp may induce antibodies against bacterial hspB which cross react with human hsp60, through the molecular mimicry of these proteins. On the other hand, it is well established that the immune response to hsp60 is closely associated with MALT lymphoma.17 At the present time, patients with gastric disease other than MALT lymphoma and elevated IgG titres to hsp60 are under careful follow-up to see whether they will develop gastric MALT lymphoma.16 If this occurs it seems reasonable to hypothesize that hspB is closely associated with pathogenesis of MALT lymphoma.

Metal acquisition proteins

Adaptation of Hp to the conditions in the gastric mucosa includes acquisition mechanisms that overcome a temporary lack of the metals iron, nickel and zinc. Iron is essential for maintaining the basic energy and redox metabolism, whereas nickel is an essential cofactor of urease, an important virulence determinant of Hp. However, as overacquisition of iron, nickel, and other metals is deleterious, the control mechanisms regulating the intracellular availability of these metals are of crucial importance.
Iron-responsive regulation in prokaryotes is usually mediated through the ferric uptake regulator (Fur) protein. Fur homologs downregulate the expression of genes involved in iron uptake when the cytoplasmatic ferrous iron concentration increases, thus abolishing iron acquisition. Iron-responsive regulation has been observed in Hp, and genetic analysis revealed that Hp possesses a Fur homolog.\textsuperscript{18} The Hp ferritin protein Pfr is a member of the nonheme ferritin subfamily, all of which store iron in the inner space of a multimeric protein shell consisting of 24 identical subunits. The protein plays a substantial role in the storage of iron and protects the bacteria from metal toxicity.\textsuperscript{19} Ferritins thus catalyze a function which is the exact opposite of that of iron uptake systems, which increase the cytoplasmic iron concentration.

**Induction of hypochlorhydria**

It is well established that acute infection is accompanied by transient hypochlorhydria.\textsuperscript{20} Suggestions for the mechanism by which Hp increases the gastric pH include: (I) presence of acid neutralizing substances (ammonia) in the infected gastric mucosa, (II) increased levels of cytokines such as IL-1b which is known to inhibit gastric acid secretion, (III) exposure of parietal cells to acid inhibitory substances released by Hp.

Hp interferes with parietal cell acid production by two mechanisms: (i) the bacterium increases proton permeability at the secretory membrane of the parietal cell (it causes back diffusion of protons from the secretory canaliculus into the cytosol of the parietal cell), and (ii) in addition inhibits H\textsuperscript{+}/K\textsuperscript{-}-ATPase activity.\textsuperscript{21}

**1.b. Factors that allow organism to evade host defense**

The bacterium possesses a well-defined battery of virulence factors that allow it to evade host defense. These are: shedding of surface proteins, catalase, superoxide dismutase, and poorly reactive lipopolysaccharide.

It has been shown that after successful colonization some bacteria are killed by the host’s defensive mechanisms, resulting in shedding of their surface proteins. These proteins are connected to receptors on the surface of other bacteria and bind cytokines and immunoglobulins. This has been interpreted as an indirect defensive mechanism of Hp to evade the host’s defenses.

Despite the fact that the organism is an obligate aerobe, it is unable to grow in atmospheric concentrations of oxygen. Microaerophilic organisms, like Hp, are particularly vulnerable to the detrimental effects of oxygen and oxidative stress. Nevertheless, they do possess some of the enzymatic machinery needed to eliminate or minimize toxic oxygen-derived products. These enzymes are superoxide dismutase, catalase, and several putative peroxidases.\textsuperscript{22}

It is well known that bacterial lipopolysaccharides (LPS) may induce both strong local and systemic inflammation in animals as well as humans, and, therefore, Hp LPS is one of the factors that could potentially influence local gastric inflammation and the clinical outcome during an Hp infection. In general, Hp LPS is much less potent in activation of inflammatory cells than LPS from members of the family Enterobacteriaceae, e.g., Escherichia coli and Salmonella spp. In spite of its relatively low toxic activity, Hp LPS has been shown to activate inflammatory cells to produce different cytokines and chemokines, such as TNF-\(\alpha\), IL-8, IL-1, and monocyte chemotactic protein-1.\textsuperscript{23} In addition, the LPS of some strains contains structures identical to the fucosylated Lewis x and Lewis y blood group antigens expressed on the gastric mucosa. The antigenic mimicry may result in immune tolerance against antigens of the pathogen or in induction of autoantibodies that recognize gastric epithelial cells, frequently observed in patients with chronic active gastritis.\textsuperscript{24}

**1.c. Factors that induce tissue injury**

**The vacuolating cytotoxin A (vacA)**

The vacuolating toxin (VacA) is a major determinant of \textit{H. pylori}-associated gastric disease. The association of vacA with peptic ulcer disease, MALT lymphoma and gastric cancer has been well validated, at least in Europe where the background population has a low incidence of type I strains (defined as cagA and vacA positive).\textsuperscript{25} Hp vacA s1 strains have been associated with the occurrence of peptic ulcer disease\textsuperscript{26} and vacA m2 allele is also associated with peptic ulcer disease and gastric cancer.\textsuperscript{27} The original hypothesis was that the s1 genotype was associated with duodenal ulcer disease and the s2 genotype had low ulcerogenic potential. Data are now overwhelming that vacA genotyping is not useful to predict symptoms, presentation, response to therapy or degree of inflammation. VacA genotyping is useful to predict cagA status.\textsuperscript{28}

The mechanism of action of vacA has recently been further described. Binding of free or membrane-bound vacA to epithelial cells is receptor-mediated. VacA forms pores in lysosomal membranes, increasing anion permeability and generating vacuoles.\textsuperscript{29} In addition, vacA has been shown to reduce transepithelial resistance by loosening tight junctions.\textsuperscript{30} Finally, vacA inhibits de novo
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Individuals infected with Hp that have a functional cag-PAI have elevated mucosal levels of IL-8, marked neutrophilic infiltration into the gastric mucosa and a theoretically increased risk of developing peptic ulcer and gastric cancer. However, in East Asia where more than 90% of isolates possess the cag-PAI, a relationship of the cag-PAI and clinical outcome has not been documented. Conversely, in Western countries, where Hp strains lacking cag-PAI are found in higher percentage, there are data showing increased likelihood of symptomatic outcome. Where the presence of a functional cag-PAI has no predictive value regarding current or future clinical presentation. Hp strains lacking a functional cag-PAI are not commensal as they are also found in patients with peptic ulcer disease or gastric cancer, only at a lower frequency.

IceA

IceA is a gene that is induced by contact with the epithelium. The gene product is unknown but it appears to be a bacterial restriction enzyme. There are two variants of the iceA gene, iceA1 and iceA2. The initial studies suggested that iceA1 was correlated with duodenal ulcer. More recent studies are conflicting. In a large study involving four different countries (U.S., Colombia, Japan and Korea), in order to avoid the regional variation of Hp genes, the results failed to confirm an association between iceA1 and clinical outcome, but a more recent large study in Japan showed that the iceA1 allele is associated with increased gastric inflammation.

2. Host – related factors

Several laboratories have provided evidence that the host response is an important determinant in Hp associated disease progress. An alternative model of Hp associated disease is the Helicobacter felis mouse model which has been extensively used to examine how the host response prevents and/or exacerbates Hp induced gastroduodenal disease. In the mouse H. felis infection model, several inbred strains of mice, exhibit severe inflammation/gastric atrophy (“high responders”), in contrast to others which are low gastritis/atrophy responders to H. felis infection. These results suggest that the nature of the host immune or inflammatory response to Hp infection in humans might be more important in determining disease outcome than Hp virulence factors.

In concordance to this hypothesis is the fact of rapid change worldwide in the incidence of gastric cancer and duodenal ulcer disease. This might be explained by an equivalent decrease in the prevalence of a particular virulence factor. However, several studies evaluating the prevalence of putative virulence factors in different birth cohorts have shown that this not to be the case.

Genetic susceptibility to infection has been documented from large epidemiological studies which implies that the host response may be regulated from genetically determined factors. There are data by developed countries, such as U.S., which exhibit different prevalence among different ethnic groups of similar socioeconomic status. Similar findings come from Southeast Asian countries in which the Malays have been shown to have a consistently low prevalence compared to the Indians and Chinese. These data show a racial-linked genetic susceptibility to infection. Genetic susceptibility has been confirmed also, in studies showing that monozygotic twins
reared apart or together had a higher rate of concordance of infection than did age-matched dizygotic twins.42

One small study has shown significant association between the prevalence of the HLA-DQ5 genotype and hp infection with accompanying atrophic gastritis or intestinal metaplasia while investigations of the HLA-DQA1*0102 genotype noted a lower prevalence of DQA1*0102 among patients with gastric cancer and co-existing hp infection. This work in HLA may be pointing in an interesting direction but requires much larger studies, adjusted appropriately for the multiple comparisons being made, before any conclusions can be drawn.43

A host-related factor which has been shown to predict disease progression is the size of parietal mass at the time of exposure to hp.44 Those with a large cell mass and high acid output have an infection confined to the antrum, where the environment is less acidic and favors hp colonization. These patients have antrum-predominant gastritis and are likely to develop duodenal ulcers. To date this unique response to hp infection has not been linked to a distinct cytokine response; duodenal ulcer disease appears to require both hypersecretion of gastric acid and the activity of proinflammatory cytokines.45

On the other hand, in those with a small cell mass, acid production is insufficient to protect the corpus from infection and subsequent cellular degeneration compromises acid output still further. This favours the loss of specialized glandular cell types, such as parietal and chief cells and the development of corpus-predominant atrophy, which appears to be a critical initiating step in the progression towards gastric cancer.45

Other host-related factors which have been shown to predict disease progression towards gastric cancer are increased gastrin levels at the time of exposure to hp,46 and single-nucleotide polymorphisms in the gene encoding IL-1b.47 It is likely that single-nucleotide polymorphisms in other genes encoding cytokines or cytokine receptors that influence the risk of gastric atrophy and cancer will be found.

3. Environmental factors

It is well established that environmental factors may also affect the clinical outcome of hp infection. For example, migrating from a region with high prevalence of gastric cancer to a region with low prevalence did not reduce the rate of cancer in the migrants but resulted in a major reduction in risk for their offspring, suggesting that the environment is more important than genetics in determining the clinical outcome of an hp infection.

The environmental factors that appear most important in determining the pattern of gastritis (and thus the risk of any of the different hp outcomes) are the presence of childhood febrile illnesses and diet.

Childhood infections such as tonsillitis, infectious diarrheas and diphtheria are associated with a marked decrease in acid secretion. Low acid secretion in childhood also occurs in malnutrition. Thus, regions where childhood infections and malnutrition are common would provide the ideal environment for hp colonization and the development of corpus-predominant atrophy, as discussed above. Diphtheria is especially prone to cause gastric damage and may even be a cause of gastric atrophy. Indeed, there is speculation that immunization against diphtheria played a major role in the prevention of early onset atrophic gastritis and therefore, of gastric cancer.48

However, in regions where childhood infectious diseases, malnutrition and hp infection are all common, one would expect a high frequency of an accelerated development of corpus gastritis. This is not a universal finding suggesting that a number of other factors may also be important in determining whether atrophic gastritis develops after hp infection. In these regions there is a year-round availability of fresh fruit and vegetables. Investigators speculated that ingestion of fresh fruits and vegetables might retard the development of gastric atrophy (the “banana hypothesis”).

There is some evidence which establish a long suspected correlation between salt intake, hp and gastric cancer risk. In the Intersalt study,49 authors note that, where measured appropriately, salt intake levels in African countries are considerably lower than in most other countries and they suggest that salt might be the permissive co-factor that is required for hp infection to act as a cancer risk factor.

Recent data suggest that some dietary habits might have anti-helicobacter activity such as the chewing of mastic gum (1 mg per day for two weeks)50 or drinking Chinese tea.51

CONCLUSIONS

Disease outcome depends on many factors, including bacterial genotype and host physiology, genotype and dietary habits. Initially, there were data showing a clear predominance of Helicobacter pylori virulence factors on human’s disease outcome but additional studies, mainly from East Asia, failed to support this model. It seems
likely that other host–derived and environmental factors are more significant in determining clinical outcome, but additional studies are needed in order to evaluate the underlying pathophysiological mechanisms involved in the clinical outcome of infection.

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