HELICOBACTER – A VERSATILE PATHOGEN
AND A VANISHING SPECIES IN THE STOMACH
(The evolution of stomach microbiology in the antibiotic era)

TORUEL WADSTRÖM

Department of Laboratory Medicine, Division of Medical Microbiology,
Lund University Hospital, Lund, Sweden

SUMMARY

*Helicobacter pylori* is a highly versatile pathogen with a coevolution of the stomach in mammals, birds and other animals. *H. pylori* is closely related to enterohepatic, bile-tolerant species (EHS), colonizing the gut of most wild rodents, felines and several other species. This evolution is complex, and the primates seem to be the environment for *H. pylori* to develop before the exodus of man from Africa about 55 000 years ago. The cagA pathogenicity island (PAI) encoding for a series of surface proteins and virulence factors (cagA to E) is unique for *H. pylori* and strongly associated to its success as a pathogen, which in turn is strongly related to a number of human diseases, i.e. type B gastritis, duodenal and peptic ulcer disease (PUD), and gastric carcinoma. Aggressive treatment strategies and screening programmes have resulted in a decline of *H. pylori* in the Western world with a concomitant decline in PUD and gastric carcinoma. The unique human-to-human transmission explains the rapid associated decline in *H. pylori*-infections in children in the Western world but not in non-industrialized societies. There, gastric carcinoma is still the second most common cancer.

Possible “non-antibiotic” strategies, such as probiotic therapy to treat *H. pylori*, and “the empty stomach” after eradication of *H. pylori* will be discussed.

INTRODUCTION

In the whole Western world and some rapidly developing Asian societies we notice a rapid change of diseases with a decline of antibiotic-curable infections and a simultaneous increase in a number of allergic diseases including allergic childhood asthma, rhinitis, eczema and other skin disorders, often proposed to be related to a “too clean” lifestyle (Figure 1) (*Chen* and *Blaser*, 2008). An infection and antigen-rich environment may be essential for maturation of a normal immune system, preventing allergies, asthma and autoimmune diseases related to disturbances of a skin- and mucosa-associated microbiota in genetically prone individuals. An infection in the gastrointestinal tract (GIT) with the gut-associated lymphoid tissues, GALT, is related to
antibiotic use and overuse worldwide (Vakil and Méraud, 2007).

*Helicobacter pylori* colonizes the human stomach shortly after weaning until recently, when modern living habits and common antibiotic use in early childhood prevent this colonization process. This fact was probably favoured by an early rotavirus and other enteric and respiratory infections. A transient pH rise in these infections may favour the gastric colonization to avoid the normal acid barrier. The impact of other factors such as diets after weaning as well as malnutrition and smoking with an alkaline stomach pH is still poorly understood (Stenström et al., 2007).

However, *H. pylori* gastritis studies in mouse and Mongolian gerbil models have been most useful to expand our understanding of the development of an acute, sub-chronic into chronic infection. *H. pylori* infections are always localized to the gastric mucosa in the stomach as well as to gastric tissues in other parts of the GIT, such as the Meckel's diverticulum (Haesebrouck et al., 2009).

We know from extensive sero-epidemiological studies that *H. pylori* appeared in the human stomach in our ancestors in Africa > 58,000 years ago (Linz et al., 2007), is present in all human populations worldwide today, and acquired early after weaning. Casswall and co-workers (1999) analysed stool samples in young children in Bangladesh and showed that a transient colonization was followed by a later infection with a seroconversion in ELISA for urease and cell surface proteins (CSP). Early infections in some paediatric populations may affect appetite and food nutrient uptake, and induce a shunting syndrome in a subgroup, probably first reported by Oderda and colleagues in a South
Italian study (Mégraud and Malfertheiner, 2010). The majority of these infections are symptom-free (i.e. silent) but induce a local and severe immune response unlike a number of transient gastric colonizing microbes from food or the oral cavity. In brief, *H. pylori* is the single and dominant member of the human stomach microbiota (Del Prete et al., 2008). It penetrates the mucus layer, colonizes the epithelium, and pass cellular tight junctions into the sub-epithelial extracellular matrix (ECM), and binds to specific components by cell surface proteins (CSP:s) and LPS such as laminin and collagen type IV (Figure 2) (McGuckin et al., 2010).

**HELICOBACTER AND MAN: A CO-EVOLUTION STORY**

**Mucus layer colonization and immune evasion**

Gastric and enterohepatic Helicobacter species (EHS) colonize the mucus layer like other micro-aerophilic microorganisms such as *C. jejuni*. However, McGuckin and co-authors (2011) emphasize that microbes such as the indigenous microflora but not pathogens, rarely penetrate to the mucin deep layer. However, a chronic colonization and immune evasion is most characteristic for *H. pylori* unlike many animal Helicobacter species, such as *H. felis* (Table 1), rarely causing a chronic infection similar to *H. pylori* gastritis. *H. pylori* has developed a strategy to minimize stimulation of Toll-like receptors (TLRs), such as TLR-5 recognizing flagellae of *Salmo-**
Table 1. Enteric Helicobacter species isolated or detected in humans

<table>
<thead>
<tr>
<th>Bacterial cultivation and/or PCR detection species</th>
<th>Cause/association in man</th>
<th>Animal host</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. canadensis</td>
<td>Gastroenteritis</td>
<td>Goose</td>
</tr>
<tr>
<td>H. winghamensis</td>
<td>Gastroenteritis</td>
<td>?</td>
</tr>
<tr>
<td><strong>H. pullorum</strong></td>
<td>Gastroenteritis, bacteraemia</td>
<td>Poultry</td>
</tr>
<tr>
<td>H. cinaedi</td>
<td>Diarrhoea, bacteraemia</td>
<td>Macaque, dog, hamster</td>
</tr>
<tr>
<td>H. sp flexispira</td>
<td>Bacteraemia</td>
<td>Pig, sheep, dog, cat</td>
</tr>
<tr>
<td>H. fennelliuae</td>
<td>Bacteraemia, septic shock</td>
<td>?</td>
</tr>
<tr>
<td><strong>H. bilis</strong></td>
<td>Cholecystitis</td>
<td>Mouse, rat, dog, cat</td>
</tr>
<tr>
<td><strong>H. hepaticus</strong></td>
<td>Chronic liver disease</td>
<td>Mouse, hamster</td>
</tr>
<tr>
<td>H. rappini</td>
<td>Cholecystitis</td>
<td>Sheep</td>
</tr>
<tr>
<td>H. canis</td>
<td>Chronic liver disease</td>
<td>Dog, cat?</td>
</tr>
</tbody>
</table>

nellae but not of H. pylori (Blaser and Atherton, 2004). TLR-9 recognizes unmethylated DNA of most bacteria but not the highly methylated H. pylori DNA which minimizes recognition. H. pylori LPS is anergic, compared to enteric pathogens, related to lipid modifications and with poor reaction in classical LPS bioassays, such as the limulus test (Moran et al., 1992; Hynes and Wadström, 2004). H. pylori LPS is camouflaged from innate immune responses on cell surfaces. However, cagA positive strains stimulate NF-κB activation in the epithelium and phagocytes with subsequent induction of pro-inflammatory cytokine production (Yanagisawa et al., 2005). The relative scarcity of H. pylori-associated disease in non-industrialized countries despite a high H. pylori prevalence may be due to predominant Th2 responses among black Africans, often chronically infected by intestinal parasites, helminths and malaria (the African enigma) (Linz et al., 2007; Del Prete et al., 2005)

Effects of H. pylori on leptin and ghrelin.

Gastric leptin levels are higher in H. pylori-infected adults than in uninfected individuals (Konturek et al., 2006). In an animal model, H. pylori infection is associated with up-regulation of ghrelin and several adipocyte genes (i.e. adipisin, resistin, adiponectin etc.), and likewise in a study from Poland (Plonka et al., 2006). Weight gain is common after H. pylori eradication, maybe related to the obesity epidemic in many Western societies and to a rapid decline in H. pylori infections (Chen and Blaser, 2008).

Chronic gastritis and carcinogenesis.

Carcinomas arise in stomachs with pangastritis (intestinal type, metaplasia, dysplasia). H. pylori-infected neutrophils release oxygen free radicals (ROS), and ascorbic acid levels are low in H. pylori-infected stomachs. Gastric cancer (GC) is number two of all cancers worldwide, increasing in African countries with an ageing population but declining in Western Europe related to an efficient eradication program (Vakil and Mégraud, 2007). The importance of dietary carcinogens in these malignancies is not well understood. However, food and snuff carcinogens act in synergy in H. pylori-infected mouse stomachs and tumours develop
regularly (Stenström et al., 2007). Well-developed mouse models are now most relevant for further studies of microbes, food and genetic background interactions in gastric cancer, similar to the more complex colon microflora-food interaction and colon cancer.

Probably all mammalian species including whales and dolphins seem to be colonized in the stomach by Helicobacter or Helicobacter-like organisms (Haesebrouck et al., 2009). However, several species studied in the early “Helicobacter history”, such as H. felis in cats and dogs, migrate freely in the mucus layer but cause no or transient mucosal penetration in contrast to H. pylori in man (Marshall, 2002). However, H. mustelae in ferrets and H. suis in piglets colonize the gastric mucosa and cause mucosal ulcers much like H. pylori with the cagA pathogenicity island (PAI) and vacuolating toxin. Candidatus Helicobacter suis is a spiral bacterium in the stomach of pigs. Experimental infections in pigs have fulfilled the Koch's postulate with lymphoid follicle proliferation and a mild inflammation mainly in the antrum close to the parietal cells, as described for H. pylori in humans (Haesebrouck et al., 2009). However, the mild infection is making this model less likely to be developed for further studies of Helicobacter/gastrospirillum pathogenesis. Some epidemiological studies in China indicate that an early H. suis infection may protect against a H. pylori infection by unknown mechanisms of interference with a lower GC incidence in the population (Haesebrouck et al., 2009).

Several outer membrane proteins (OMPs) have been identified, like mucus-binding molecules including a neutrophil binding and activating protein, or HPNAP (Parker et al., 2010). In this H. pylori OMP gene family (Oleastro et al., 2010) single or two copies of the homA/homA, homA/ homB, or homB/homA and homB were found to be associated with gastro-duodenal disease with gastric inflammation and stomach atrophy, and identified as tissue adherence factors and implicated in IL-8 secretion. On the opposite, the homA gene is not associated with gastric inflammation.

H. pylori displays the highest level of genomic variability in bacteria with a high frequency of recombination contributing to the great diversity (Linz et al., 2007). Intragenomic recombination in the OMP genes occurs during a chronic H. pylori infection, probably reflecting a selective pressure for adhesins to adapt to the individual host and variations in the immune responses. H. pylori polymorphism reflects human phylogeography and human migrations, a most reliable marker of host pathogen co-evolution, facilitated by the long-term contact between the infecting strain and the host. Resulting genetic combinations seem to be critical for the ecological success of H. pylori strains (Oleastro et al., 2010). This great complexity in antigenic variations is a great challenge for H. pylori vaccine research.

**H. PYLORI AND ANTIBIOTIC RESISTANCE**

Suboptimal antibiotic eradication of H. pylori with up to a new quadruple therapy to treat patients to eradicate H. pylori upon several therapy failures confirmed by urea breath testing (UBT), gastroscopy or serology is a new challenge to develop alternative therapies (Vakil and Mégraud, 2007).

Cazzato and co-authors (2004) proposed to combine an antibiotic regime with a probiotic, i.e. Lactic Acid Bacteria (LAB) treatment to prevent side ef-
fecteds such as gastric reflux disease. Sakamoto and co-authors (2001) characterized a probiotic strain of Lactobacillus gasseri, and developed a Japanese yoghurt at the Meiji company to suppress H. pylori stomach infection as shown by UBT. Michetti and co-workers (1999) showed by UBT that another probiotic strain of LAB, L. acidophilus La1, inhibits the infection. Interestingly, LAB in the murine fore stomach inhibits the H. pylori infection in C57 Black and BALB/c mice (Wang et al., 1997), while germfree mice are more easily infected by a cagA positive strain of H. pylori devoid of LAB microflora in the upper stomach.

LAB and Bifidobacteria produce a number of antimicrobial activities (AMA), including peptides, which may inhibit H. pylori as well as a number of other pathogens such as Clostridium difficile (Ljungh and Wadström, 2009). This may be important in the future with a rapid increase in antibiotic-associated diarrhoea (AAD), sometimes as a severe complication upon a H. pylori antibiotic eradication therapy including clarithromycin or a quinolone (Szajewska et al., 2009). Thus, probiotics may be a useful adjunct with increased rates of eradication with an increase of 77% to 80% in one study (O'Connor et al., 2010).

THE STOMACH – AN EMPTY MICROECOLOGY NICHE AFTER H. PYLORI ERADICATION

An increase of gastro-duodenal reflux disease, or GERD, occurs in all Western societies today, associated with a rapid decline H. pylori infections in children varying from 33% to 7% in some epidemiological studies (Figure 1) (Chen and Blaser, 2008). One study demonstrated that H. pylori is 600 times more abundant in vomit samples compared with stool samples, and that vomiting is an important factor for transmission among children, e.g. in day care centres.

THE EVOLUTION OF H. PYLORI, OTHER GASTRIC AND ENTERIC HELICOBACTER SPECIES

A few years before Marshall and Warren published the Nobel Prize winning original reports in 1983 on H. pylori as the cause of type B (“bacterial”) gastritis (Table 2) while H. felis and other related spiral-shaped organisms colonize rodents, cats and dogs (Table 1) (Haesebrouck et al., 2009). Studies in monkeys and other animals have confirmed that H. pylori is a unique human pathogen. The closest species to H. pylori may be a gastric species reported in beluga whales and dolphins (Haesebrouck et al., 2009). Genetic analyses of H. pylori in various populations confirmed a close relation to the human exodus from Africa 58,000 years ago (Linz et al., 2007). The great number of newly discovered species and new species “in the pipeline” of enteric Helicobacter in wild and laboratory mouse colonies suggest a complex evolution from unknown ancestor(s) closely related to Wollinella (Table 1).

Eradication of H. pylori from the human stomach may be in close analogy to another strictly human pathogen, i.e. the smallpox virus. However, the drastic fall in colonization rates in
Table 2: The discovery of Helicobacter pylori

<table>
<thead>
<tr>
<th>Year</th>
<th>Author(s)</th>
<th>Discovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1893</td>
<td>Bizzozero</td>
<td>Spirochetes in dog stomach</td>
</tr>
<tr>
<td>1906</td>
<td>Krienitz</td>
<td>Spirochetes in the stomach with gastric cancer</td>
</tr>
<tr>
<td>1917</td>
<td>Dragstedt</td>
<td>Bacteria do not induce gastric ulcer</td>
</tr>
<tr>
<td>1938</td>
<td>Doenges</td>
<td>Spirochetes induce gastritis in monkeys and humans</td>
</tr>
<tr>
<td>1979</td>
<td>Warren</td>
<td>Spiral bacteria in the human stomach</td>
</tr>
<tr>
<td>1983</td>
<td>Marshall</td>
<td><em>H. pylori</em> isolated and cultured</td>
</tr>
</tbody>
</table>

young children in US, EU and other highly developed human societies today will never reach zero, as for smallpox. Moreover, we never experienced an “empty” sterile human mucosal surface. We know from studies in obesity patients before and after gastric bypass operations that oral microbes as alpha streptococci and enteric organisms (as various enterococcal species) can cause overgrowth in the stomach in chronic type A and B gastritis. A few new *Lactobacillus* species, like *L. kalixanda* (Roos et al., 2005) in gastroscoped human volunteers, and a few acid-tolerant *L. salivarium* and *L. reuteri* were reported recently.

It seems likely that we can develop a new probiotic concept to encourage use of stomach-adaptable non-virulent microorganisms to fill this empty niche. Since the borderline plastic pathogen and *H. pylori* probably now continues to decline and disappear it seems likely that a new concept discussed by B Marshall to develop a non-virulent attenuated *H. pylori* strain as a vaccine delivery system may also become a future probiotic candidate in analogy with *E. coli* strain Nisslè in ulcerative colitis (Ljungh and Wadström, 2009).

Finally, studies on the stomach microbiota should continue to explore the pathogenic properties in pigs (*H. suis*) and the complex gastric microflora in dogs and cats. The fore-stomach of these animals as well as of horses and rodents are colonized with LAB (Haesebrouck et al., 2009). These should be eradicated to permit a successful Helicobacter experimental infection (Wang et al., 1997).

HELICOBACTER AND CANCER

Marshall and Warren already in 1983 proposed that *H. pylori* was causing chronic type B (bacterial) gastritis and was an important vector in the development to atrophy and gastric metaplasia to dysplasia and gastric cancer, or GC (Marshall, 2002). The development of combined proton pump inhibitors’ (PPI) and antibiotic therapies now rapidly changed the GC epidemiology in societies all over the world. With a rapid decline in *H. pylori* infections in early childhood the person-to-person spread is rapidly declining. Without a chronic *H. pylori* gastritis at least 80-90% of GC will be prevented, previously calculated to occur in about 11% of *H. pylori* type B gastritis, thus a stronger
cancer risk factor than smoking in lung cancer (Stenström et al., 2007). Since most H. pylori infections are silent, a strategy to screen for GC with serology and gastroscopy with histopathology and culture, and PCR diagnosis of H. pylori infections are discussed, most recently at the European H. pylori conference in Florence, November 2010 (see: www.helicobacter.org).

In brief, it seems most appropriate to develop a pan-European screening program to diagnose H. pylori infections in our aging population with special attention to high H. pylori infection rates in South and East Europe and in immigrants from Turkey and other countries in the Middle East and in Africa. GC is still number four of all cancers worldwide and increasing in Indonesia and some other Asian countries with a rapidly growing young population, living in poor sanitation conditions. Interestingly, Canada decided already a decade ago to perform H. pylori serology on all immigrants applying for a Canadian citizenship (R. Hunt, McMaster University, personal communication). Thus, a H. pylori vaccine and other prevention methods seem to be a most attractive strategy to fight peptic ulcer disease and stomach cancer in Asia and Africa. B Marshall's laboratory in Perth is developing a new vaccine concept based on an attenuated H. pylori strain as a candidate to carry other vaccine candidates for an oral to stomach vaccine delivery.

HELICOBACTER, ANIMAL MODELS AND CANCER

The development of models to study H. pylori infection in mice and Mongolian gerbils are important tools to evaluate vaccine candidates and other strategies to prevent and treat H. pylori and other Helicobacter infections (see below). A number of dietary components such as antioxidants suppress these infections, and a low antioxidant diet enhances them. Recently, also some EHS, such as H. bilis, H. pullorum and H. hepaticus were shown to cause a chronic gut, biliary tree and liver infection in specific mouse models as the IL-10/- mouse. We isolated H. ganmani in a spontaneous outbreak of IBD-like disease in IL-10 -/- mice with a histopathology similar to ulcerative colitis and sclerosing cholangitis in humans (Nilsson et al., 2008). These findings are also supported as new disease syndromes in a mouse model to study bile stone chronic cholecystitis in hyperlipemic mice (Maurer et al., 2005). Likewise, they support early observations of chronic cholecystitis related to a Salmonella and C. jejuni biliary tract infection, probably very common in many non-Western societies. A possible link between Helicobacter infections and chronic cholangitis and bile stone disease is under investigation in several laboratories (Nilsson et al., 2005; Pandey et al., 2009; Karagin et al., 2010).

More research on a relation between EHS, biliary disease and IBD is certainly needed!

ACKNOWLEDGEMENT

Original research cited was supported by grants from the Swedish Medical Research Council and of Lund University Hospital ALF grants through more than two decades.
LITERATURE


Nilsson, H-O., Pietroiusti, A., Gabrielli, M., Zocco, M.A., Gasbarrini, G., and Gasbar-


