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HELICOBACTER: CHRONIC EFFECTS AND ROLE IN HOST MICROECOLOGY

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SUMMARY

Helicobacter pylori is the first named species of the Helicobacter/Wolinella family, now including more than 20 species and about 10 candidate species. The organisms are all micro-aerophilic "mucinophiles" with a few exceptions. *H. pylori* is the prototype for a number of bile-sensitive species colonising the stomach of most mammals, including dolphins and whales. The low toxicity of the lipopolysaccharide (LPS) and a number of properties unique for these species determine how they may cause life-long infections. *H. pylori* carries the vacA toxin as well as a set of other virulence traits permitting optimal early colonisation of the host, e.g. in childhood. The cagA pathogenicity island (PAI) makes $cagA^+$ strains of *H. pylori* more virulent than cagA- strains to develop chronic active gastritis, gastric atrophy and pre-cancerous lesions in the host as well as in mouse and mongolian gerbil models. *H. pylori* as well as a number of entero-hepatic bile-tolerant species are camouflaged from the innate immune system of the GI epithelial cell surfaces, yet $cagA^+$ *H. pylori* transcribe NF-κB to the nucleus of these cells and of macrophages and other cells. At least *H. pylori* evades the host immune system by a number of responses such as molecular mimicry of the H–K adenosine triphosphatase and of gastric cell surface fucosylated antigens. The degree of inflammation is modulated by the IL-1β cytokine polymorphism and probably by a number of other host factors.

The co-evolution of *H. pylori* and man back to the origin of mankind is clearly defined with a sophisticated haemostasis between the *H. pylori* as a pathogen. Alternative scenarios in the 21st century in several parts of the world with a "clean" *Helicobacter*-free human stomach are addressed as well as recent reports of a newly discovered gastro-oesophageal microflora and the rapid increase in GERD, Barrett´s oesophagus, oesophageal cancer and obesity as well as changes in living conditions in Western societies.

INTRODUCTION

Helicobacter pylori lives in the mucus layer overlying the gastric epithelium and does not appear to invade tissues. However, the mucosa underneath the area of colonisation is invariably inflamed (chronic superficial gastritis; *Northfield* et al., 1994). Most infected persons do not show clinical manifesta-

Table 1: Entero-hepatic bile-tolerant *Helicobacter* species

Species	Comment
Helicobacter pylori	some strains are bile-tolerant
Helicobacter pullorum	common in chicken
Helicobacter bilis	common in rodents
Helicobacter hepaticus	common in rodents
Helicobacter cholecystus	common in hamster
Helicobacter canis	common in dogs
Helicobacter rappinii	certain subtype common in sheep
Helicobacter ganmani	anaerobic

tions of the inflammation. Studies that include human volunteers, experimental animal infections and treatment of patients with antimicrobial agents show that *H. pylori* plays a critical role in this inflammation and in these diseases. Much evidence suggest that *H. pylori* is an indigenous microbe of the human stomach and that most, if not all, mammalian species harbour related *Helicobacter* species with a long co-evolution of microbe and host (*Blaser,* 1998; *Richter,* 2001). *H. pylori* probably evolved from bile-tolerant enteric *Helicobacter* species colonising rodents and other mammals, including primates and man (*Fox* et al., 2001; Tables 1 and 2). The phylogenetic tree of proteobacteria includes *Sulphurospirillum*, *Arcobacter*, *Campylobacter, Helicobacter* and *Wolinellae* (*On*, 2001; Figure 1).

More than 20 species of *Helicobacter* are recognised today, with *H. heilmanii* as a second gastric species. This species and some others are highly fastidious and difficult or impossible to culture *in vitro* under micro-aerophilic or anaerobic conditions with *H. ganmani* as the prototype of the second group (*Robertson* et al., 2001). All species are highly motile and possess non-sheathed or sheathed flagellae enabling them to swim in the mucin layer (*Andersen* and *Wadström*, 2001). Suerbaum and colleagues (*Schreiber* et al., 2004) recently showed that *H. pylori* prefers a specific part of the gastric mucin layer, probably regulated by acid secretion, *H. pylori* urease and ammonia formation, a metabolite most toxic for the gastric mucosa. Urease-negative as well as catalase-negative mutants are unable to colonise and infect the mouse stomach, suggesting that ammonia production and the redox potential are crucial to initiate the infection. Several ge-

Table 2: Evidence that *Helicobacter pylori* infection of humans is of ancient origin.

- Extensive genetic heterogeneity
- Acid-secreting stomachs arose early (300 million years ago!) in vertebrates
- *Helicobacter* genus is highly prevalent in the stomach and gut of all vertebrates?
- *H. pylori*-like organisms are widely present in the stomach of primates
- High incidence among human populations in Asia and Africa of *H. pylori* (>80-90%)
- *H. pylori* is adapted to persist for lifetime in the human stomach

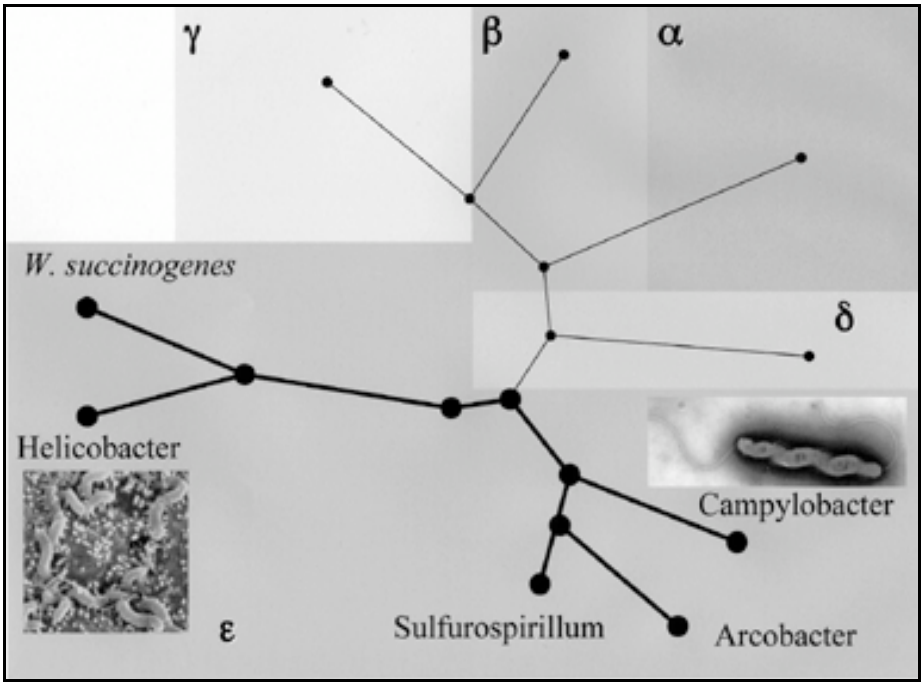

Figure 1: Representation of the phylogenetic tree of proteobacteria (modified from *On*, 2001).

netic studies of *H. pylori* isolates from a single human stomach show that these microbes are highly adaptive organisms, which partly explains that this pathogen can persist for decades in a single stomach inducing a low grade tissue inflammation (*Blaser* and *Atherton,* 2004). This adaptation involves mutations and recombination, and many strains may be classified as hypermutation phenotypes. *H. pylori* is able to maximise diversity of genetic sequences under strong selective pressure while maintaining alleles critical for its lifestyle (*Björkholm* et al., 2004).

Helicobacter-like organisms, resembling the syphilis spirochete, were reported by several pathologists in human and animal stomachs already in the period from1880 to 1890, including beautiful studies in dogs by Bizzozeroni in Italy, describing a species today named *H. bizzozeron* (*On,* 2001). However, its possible role as a gastric pathogen and not a post mortem "by-stander" was not addressed properly until *Marshall* and *Warren* (1984) in 1982 grew the first *Campylobacter pyloridis* (later *C. pylori* and renamed to *Helicobacter pylori* in 1989). By drinking viable *in vitro* cultured *H. pylori* cells, Marshall and colleagues showed that it induced acute achlorhydria and dyspepsia, which was suppressed or cured by a bismuth-antibiotic therapy.

Later, *in vitro* co-culture studies of *H. pylori* and gastric cancer epithelial (AGS) cells showed that strains containing the 35 to 40 kilobase cag pathogenicity island (PAI) flanked by specific 39 basepair direct DNA repeats induced a higher cytokine response (IL-8), and promoted an anti-apoptotic pathway aiding persistence of the organism in the gastric mucosa (*Crabtree,* 2001).

Another reason for its persistence is the molecular mimicry, in part due to the low biological activity of its lipopoly-

saccharide (LPS)(*Moran* et al. 2000*; Blaser* and *Atherton,* 2004). Molecular mimicry between *H. pylori* antigens and H^+,K^+-adenosine triphosphatase activates $CD4^+$ T cells in the stomach. This leads to gastric autoimmunity in genetically susceptible individuals via molecular mimicry (*Amedei* et al., 2003).

H. PYLORI PATHOGENESIS – A MULTIPLE STEP INFECTION TO CHRONIC GASTRITIS AND GASTRIC ATROPHY

Early development of mouse models has clearly given good opportunities to elucidate the *H. pylori* pathogenesis, and to develop alternative prophylactic and treatment schedules to standard proton pump inhibitor (PPI) and antibiotics (*Hamilton-Miller*, 2003). Mice given the vacuolating (vac) toxin orally developed ulcers. However, strains producing a vac toxin with an S1/m2 mid-region seem to bind poorly to specific cell lines and induce less tissue damage and cell membrane pores (*Blaser* and *Atherton*, 2004). Moreover, the S2 genotype is associated with a lack of the cag PAI and may induce a less severe gastric inflammation. Transient oral and gastric *H. pylori* colonisation occurs in children, as shown in a study from Dhaka, Bangladesh (*Casswall* et al., 1999). It is likely that *H. pylori* is a paediatric infection, "achieved" soon after weaning in all primitive societies (*Blaser,* 1988). Weaning habits such as maternal chewing of food and early rotavirus and other viral infections changing the gastric physiology influence the time of acquisition. Ongoing infection can be detected by faecal immunomagnetic bead based PCR or antigen detection methods (*Weingart* et al., 2004). A humoral as well as local immune response is rapidly induced. Antibody titres remain for several decades but interestingly, $cagA^+$ strains disappear more rapidly (*Perez-Perez* et al., 2002).

The *H. pylori* LPS is an anergic low toxicity endotoxin with a unique lipid A core structure (*Hynes* and *Wadström*, 2004). It stimulates only macrophage Toll-like receptor (TLR4) and not gastric TLR4 (*Bäckhed* et al, 2003). CagA-positive strains induce transcription of NF-κB in the epithelium through recognition of Nod1, an innate intracellular pathogen-recognition molecule, recognising soluble bacterial peptidoglycan fragments (*Kim* et al., 2004). How such molecules as well as other cell surface, extra-cellular and cell lysis molecules, including nucleic acids, are delivered to the gastric mucosa is poorly understood. Further studies are needed to define new possible interventions, such as probiotic-based strategies including anti-*Helicobacter* peptides and bacteriocins (*Hamilton-Miller*, 2003; *Lorca* et al., 2001).

The *H. pylori* infection down-regulates the immune response, suppresses T-cell proliferation and induces selective T-cell apoptosis (*Shirin* and *Moss,* 1998; *Lundgren* et al., 2003). The early gastric colonisation involves a Lewis B binding cell surface (HOP) protein as well as a number of other adhesins, such as sialic acid lectins (SAL´s) recognising cell surface mucin and glycolipid molecules (*Gerhard* et al., 2001; *Falk* et al., 2000). Inhibition studies with milk glycoconjugates (*Hirmo* et al., 1998; *Wang* et al., 2000a; *Wang* et al., 2001) and a probiotic strain of Lactic Acid Bacteria (LAB) could prevent, suppress or cure *H. pylori* infection in a mouse model (*Cruchet* et al., 2003).

H. pylori further induces a rapid, early neutrophilic activation by a specific molecule (HPNAP) (*Teneberg* et al., 1997). This induces a rapid cell uptake

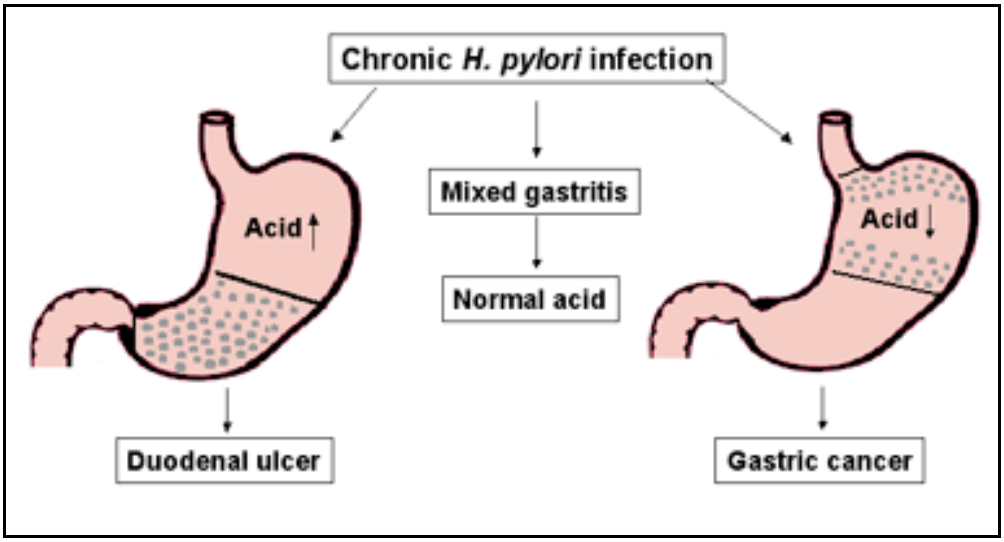

Figure 2: Divergent responses to *Helicobacter pylori* infection.

through lectino-phagocytosis by SAL´s and glycosaminoglycan (GAG) surface lectins. Other chronic infections, such as a specific helminth or parasite infection may modulate the Th1/Th2 immune response to a predominant Th2 response in black Africans, "the African enigma". This may reflect a genetic predisposition selected by malaria (*Fox* et al., 2000; *Bennedsen* et al., 1999).

A specific IL-1β polymorphism induced by *H. pylori* increases the risk of severe gastritis proceeding to gastric atrophy, hypochlorhydria and adenocarcinoma (*Blaser* and *Atherton,* 2004; Figure 2). Polymorphism of the TNF-α and IL-10 genes may have a similar modulating effect on the outcome of a chronic inflammation after one or two decades. A sophisticated somatostatin regulation of gastrin release, a growth factor for *H. pylori,* creates a feedback loop reversal after curing of an *H. pylori* infection (*Zhao* et al., 2003). A persistent increased tissue gastrin level increase the parietal cell mass and enhances the process of gastric metaplasia in the duodenum associated with *H. pylori* inflammation and duodenal ulcer disease (*Wang* et al., 2000b).

H. PYLORI IN THE 21ST CENTURY

H. pylori still infects the majority of children in the non-industrialised world, leading to pangastritis and stomach atrophy. Depending on food intake, i.e. a high or low level of fruit, antioxidants and possibly food carcinogens, the risk of gastric malignancies varies between 2.7 and more than 12-fold in various studies with high prevalences in Japan, Northern-China, the Baltic countries and other parts of Eastern Europe (*Forman* and *Graham,* 2004). However, in Western societies with a low incidence of *H. pylori* infection in children (< 2% today in Scania, Southern Sweden), the human stomach homeostasis and health should be studied since pangastritis leads to a reduction of gastric acid production (*Sande* et al., 2001; Figure 2). It is likely that an increased acid production is

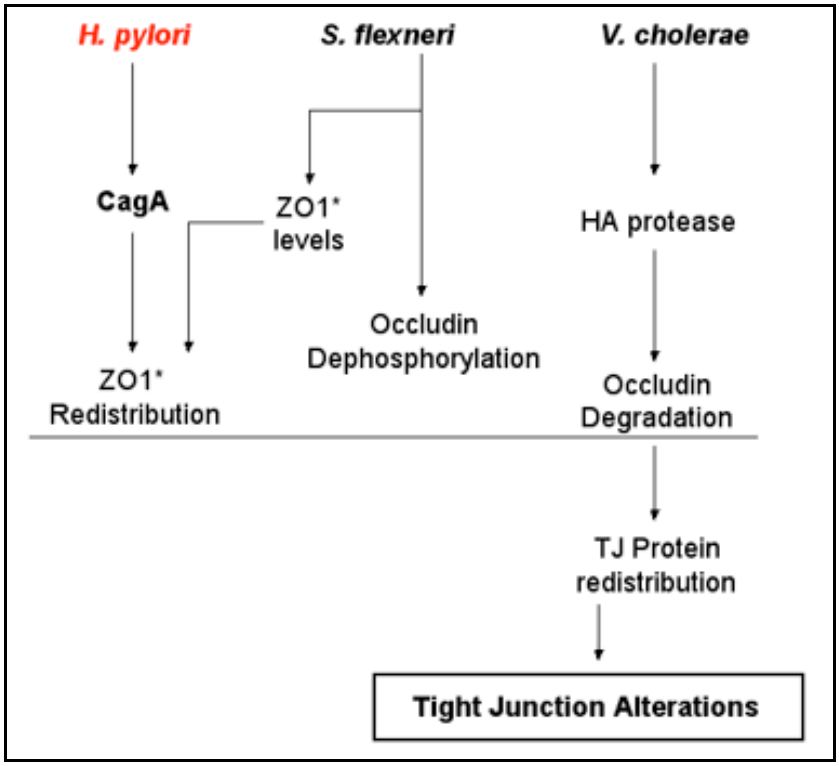

Figure 3: Disruption of tight junctions by microbes and microbial products.
*: ZO1= zona occludens 1.

associated with GERD-reflux oesophagitis and related conditions, such as Barrett´s oesophagus and pre-malignant epithelial changes (*Fitzgerald,* 2001).

Moreover, *H. pylori* infection is associated with elevated serum leptin levels (*Breidert* et al., 1999; *Matarese* and *Lechler,* 2004). A weight gain is common after *H. pylori* eradication (*Azuma* et al., 2001), possibly predisposing to adult as well as adolescent obesity. A high intake of antioxidant-rich food and food supplements can inhibit free reactive oxygen metabolites (ROM´s) and inhibit transcription of NF-κB and DNA mutations in the epithelium (*Wang* et al., 2000b). The relative role of food carcinogens, such as nitrosamines and water rich in nitrates, in gastric carcinoma development should be studied in various geographical regions of the world. Likewise, in patients on a long-standing PPI regime to suppress acid reflux (GERD) disease, gastric overgrowth by enteric microbes with potential carcinogen production (c.f. enterococci) should be investigated.

THE IL-10 -/- MOUSE AND *HELICOBACTER*-INDUCED GASTRITIS AND COLITIS

LAB of the upper mouse stomach form a barrier towards *Salmonella, Helicobacter* and other bacterial gastrointestinal pathogens. An early germ-

free (GF) mouse model to study anti-*Helicobacter* effects of *L. gasserii* was developed in Japan (*Kabir* et al., 1997). More recent studies indicate that GF mice are not readily colonised by *H. pylori* and enteric *Helicobacter* sp. (*E. Norin, H.-O. Nilsson,* and *T. Wadström,* unpublished observations). However, an IL-10 -/- mouse derived from C57-black mouse responded to *H. pylori* with a more severe gastric inflammation than Balb-c, and this mouse strain seems promising to optimise a mouse *H. pylori* gastric cancer model (*Kullberg* et al., 2003).

The IL-10 -/- mouse is susceptible to *H. pylori* as well as to natural and experimental *H. hepaticus* and other enteric *Helicobacter* species (*H. bilis, H. ganmani,* etc., see Table 1).

A first *H. hepaticus* colitis study in IL-10 -/- mice by Pena and co-workers (*Pena* et al., 2004) suggests that this model may become the model of choice to study effects of probiotic microbes as well as other therapies towards gastric and enteric *Helicobacter* infections. IL-10 is associated with several traits such as gut permeability regulation, which seems important for LAB as well as antioxidant anti-*Helicobacter* effects. Similar mechanisms were proposed for *Salmonella* and other enteropathogens, including *Vibrio cholerae* (Figure 3). The complete genome of *H. hepaticus* has been published (*Suerbaum* et al., 2003). This will provide a valuable tool to identify virulence genes.

However, in the near future well defined conditions to create and keep *Helicobacter*-free mouse colonies should be addressed, including a modified Schaedler flora to stimulate studies on chronic experimental models of inflammatory conditions and to avoid interference of murine *Helicobacter* induced inflammations in various experimental models. These include inflammatory bowel disease (IBD) in dextran sulphate and other chemically as well as microbe induced IBD-like syndromes.

CONCLUSIONS

The gastric as well as the intestinal epithelium is an interactive barrier that directs neutrophil movement. Specific peptides act via Toll-like receptors and induce NF-κB transcription with production of pro-inflammatory cytokines. *H. pylori* and several enteric *Helicobacter* species may disrupt tight junctions (TJ:s) (Figure 4) in a similar way as discussed for enteric pathogens such as *Shigella flexneri*. Ongoing studies in several laboratories aim at means to stabilise TJ:s, e.g. by probiotic and antioxidant treatment. This may also be an important step in inflammatory bowel disease (IBD) research in IL-10 knockout mice as well as in human patients. Further comparative studies on the pathogenesis of *H. pylori* and enteric IBD-inducing species (*Sturegård* et al., 2004) may reveal new preventive and curative methods for chronic gastric and enteric inflammations.

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