HELICOBACTER PYLORI: CURRENT STATUS AND FUTURE PROSPECTS

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ABSTRACT

*Helicobacter pylori* is a global pathogen that causes severe gastrointestinal diseases leading to a significant morbidity and mortality. There is an effective treatment for peptic ulcer disease, however, this is being compromised by an increase in the prevalence of antibiotic resistance. Although alternative rescue regimens have been advocated, the best strategy would be to prevent disease, especially in the case of gastric cancer for which there is still no treatment. One approach is to inhibit the first step in the pathogenic process- adhesion of the organism to the host tissue. Another and probably a better approach is vaccination, but clinical trials have so far been unsuccessful. There is still a large uncertainty in relation to how *H. pylori* causes disease. Knowledge from genomics, proteomics, and the relationship between polymorphism of the bacterium and the host, as well as the continuing investigation of the role played by important virulence factors in the outcome of the disease, will help both in understanding pathogenesis of disease and in the design of the best vaccine.

Key words: *Helicobacter*, peptic ulcer, gastric cancer, management, adhesion, genome, proteome, VacA, CagA
1. INTRODUCTION

*Helicobacter pylori* emerged into prominence in 1983 when it was first isolated from the human stomach by Warren and Marshall\(^1\). Warren believed it was the cause of gastritis and gastric ulcers and tentatively suggested this. This suggestion was met with disbelief by the majority of the medical profession as a paradigm was already in place to explain ulcer development - too much spicy food, too much smoking and too much acid. At that time, the treatment was surgical - selective vagotomy or partial gastrectomy. With time and accumulating evidence, there was a paradigm shift and currently the majority of the medical profession now believes that *H. pylori* is the principle cause of gastric ulcers - and more besides. The treatment now is a short course of antibiotics combined with anti-acid medication.

*H. pylori* is a micro-aerobic gram negative bacillus that is found in over 50% of the world’s population, making it one of the commonest infections. It is acquired in childhood although the route(s) of transmission remain undefined\(^2\). The organism has a highly variable genetic make-up, which underpins the relationship between the type of the infecting strain and the clinical outcome. Type I strains carry an assemblage of virulence markers and are more likely to be found in patients with disease than are Type II strains, which are relatively less virulent. This genetic heterogeneity can also be used to identify separate variants of *H. pylori* and these variants are geographically distributed according to ethnic background and historically recognised population migrations\(^3\). Although the first *Helicobacter* to be isolated, *H. pylori* is now only one member of the Genus, as similar species have been isolated from a range of animals as diverse as the cheetah, the tamarin, the mouse and the dolphin\(^4\).

The majority of individuals colonised by *H. pylori* are asymptomatic although histologically they will have gastritis. A proportion of patients will develop peptic
ulcers (duodenal and gastric ulcers) and a smaller proportion will develop gastric cancer. Globally *H. pylori* is the major cause of gastric cancer and has been classified as a Class I carcinogen by the WHO. In duodenal ulcer, the organism is found principally in the antrum of the stomach and the acid load in the stomach, induced by the presence of the organism, is high. In gastric ulcer, the converse is true - the organism is not confined to the antrum but can be found throughout the stomach (antrum and fundus) and the amount of acid in the stomach is low. This latter finding is due to death of the acid producing cells that are found mainly in the fundus of the stomach, caused by the presence of the organism. Conditions of persistent inflammation with low acid in the stomach, along with various other co-factors, such as a diet poor in vitamin C, may eventually lead to gastric cancer.

In order to understand the diseases caused by *H. pylori*, it is necessary to understand the host/pathogen interaction. However, a complete understanding of the pathogenesis of disease is still a long way off, although a clearer picture of the manifold effects of *H. pylori* on the host and the contribution of the host to development of disease is slowly emerging. *H. pylori* Type I stains possess a gene called cagA - a virulence marker and an indicator for the presence of a Cag pathogenicity island (PAI). This is a collection of about 30-40 genes, which are necessary for the synthesis of a Type IV secretion apparatus. Virtually, all strains also possess a gene, which synthesises a vacuolating cytotoxin - vacA. However, only Type I strains secrete the vacA cytotoxin. Type II strains lack cagA and do not secrete vacA. Type I strains are associated with more severe gastroduodenal disease compared to type II strains.

*H. pylori* has both direct and indirect effects on the host, which eventually lead to the development of disease. The helical shape of the organism facilitates its penetration of the mucus layer covering the gastric epithelium. The next step in the infection is its
ability to bind to gastric epithelium. The organism expresses a number of ligands through which it binds to the host cells. Once bound, *H. pylori* expresses a number of enzymes that affect the quality of the mucus barrier and directly damage the host cell. Urease expression results in hydrolysis of urea and a release of ammonia, which disrupts the mucus layer and has a direct cytotoxic effect. The organism also secretes phospholipase, which further disrupts the mucus layer. The loss of this protective barrier allows the stomach acid and digestive enzymes to have direct access to the gastric epithelium. In addition, Type I strains will secrete VacA-and the cagA protein will be injected into host cells, which will result in further damage to the host epithelium, and ultimately will induce the release of chemotactic cytokines, which recruit inflammatory cells to the area. The release of reactive oxygen species and cytotoxic proteins from the inflammatory cells leads to additional damage to the host epithelium. *H. pylori* also has indirect effects on the host that also lead to damage. As the host mounts an acquired immune response to the presence of the organism, antibodies are produced that cross-react with host cells and these can induce antibody mediated cell death. Finally, by interrupting the negative feedback loop that regulates the amount of acid produced by the stomach, very high levels of acid may cause further damage to the gastric epithelium that is now unprotected by the mucus layer that covers it.

In addition to microbial virulence factors, both host and environmental factors contribute to disease. Within the host, certain polymorphisms related to cytokine production and acid secretion are associated with more severe disease compared to other host polymorphisms. Most severe disease occurs when certain microbial virulence markers (e.g. cag, vac) are present in a susceptible host genetic background. Environmental, host and microbial factors implicated in *H. pylori* disease are
summarised in Table 1.

As mentioned earlier, *H. pylori* is a global pathogen that causes severe gastroduodenal diseases i.e. peptic ulcer disease and gastric cancers, leading to a significant morbidity and mortality. Although peptic ulcer disease is treated by a short course of antibiotics combined with anti-acid medication, there is a worrying increase in the prevalence of antibiotic resistance. Moreover, when gastric cancer is diagnosed, it is often too late. There are several strategies to overcome these problems. Several alternative rescue regimens have been advocated to treat peptic ulcer disease. Additionally, there is increasing interest in the role of host polymorphisms related to drug metabolism and how these polymorphisms affect success of therapy. One of the best strategies would be to prevent disease by using a vaccine. However, clinical trials have been unsuccessful. One novel approach is to inhibit the first step in the pathogenic process-adhesion of the organism. Although a considerable amount of knowledge has been acquired since its first isolation, there are still large areas of uncertainty in relation to how *H. pylori* causes disease. A key area of current interest is host/pathogen interaction and the mechanism of disease causation. Knowledge of the genome sequence of *H. pylori* and the development of molecular techniques, such as microarrays, have allowed expression of bacterial genes during an actual infection to be investigated. Additionally, this information can also be used to identify potential vaccine candidates and to investigate evolution of the organism and its interaction with its environment. The relationship between polymorphism in the bacterium and the host- and how they interact to enhance disease potential are reviewed in the case of gastric cancer. Moreover, over the past year, other roles for VacA and cagA, two of the most important virulence factors, have been discovered, which will increase our
knowledge of pathogenesis. Finally, although *H. pylori* is related principally to gastroduodenal disease, there are many other species of *Helicobacter* from a whole range of different animal species. These non-pylori *Helicobacter* species are of engaging interest because of their possible relationship to the pathogenesis of inflammatory bowel disease and liver cancer. Certainly, some of these *Helicobacter* species can induce colitis and liver cancer in rodent models of infection and the question is whether they may be related to human forms of these two diseases.

2. MANAGEMENT OF PEPTIC ULCER DISEASE

The major group of individuals in which eradication of *H. pylori* is undertaken, and for which there is a clear benefit, is with peptic ulcer disease. The currently accepted management for the eradication of *H. pylori* is a proton pump inhibitor combined with two antibiotics: clarithromycin and either amoxycillin or metronidazole. Important parameters in determining whether eradication treatment for *H. pylori* is effective, is the prevalence of antibiotic resistance and increasingly it is recognised that host polymorphism is also a factor.

Antibiotic resistance is increasing and is currently high in some areas of the world. Metronidazole resistance is more common than clarithromycin resistance but both have an adverse effect on the eradication rate. Second line therapies following failure of one of the initial regimes have included triple or quadruple regimens containing antibiotics such as levofloxacin or furazolidone. Rapid methods of determining microbial resistance, so that appropriate targeted regimens can be given at the outset, are urgently required.

The genotype of the host, with respect to the ability to either extensively or poorly metabolise (and thus inactivate) proton pump inhibitors (locus P4502C19) has been
suggested as important in pharmacoeconomics when giving standard eradication therapy. Two phenotypes are recognised: extensive metabolizers of proton pump inhibitors (homozygous and heterozygous genotypically) and poor metabolisers. In a study comparing genotyping or not genotyping prior to eradication therapy, a clear cost advantage was shown for genotyping. The eradication rate for the two groups, extensive versus poor metaboliser, has been calculated in another study in which a standard eradication regimen was given and the patients genotyped. Failure to eradicate *H. pylori* was largely confined to those with the extensive metaboliser phenotype of CYP2C19. A compounding effect of IL1-β polymorphism on the cytochrome P4502C19 genotype affecting the eradication rate, has also been shown in a study of 249 patients with peptic ulcer disease. Lower eradication rates were achieved in the normal acid secretion IL1-β group if the subjects were of the extensive metaboliser phenotype (60%) compared to the poor metaboliser group (95%). This difference was lost in the low acid secretion IL1-β polymorphism and eradication rates were independent of the CYP2C19 genotype.

3. NEW STRATEGIES TO FIGHT HELICOBACTER PYLORI INFECTION

3.1 Antimicrobial agents

In addition to new combinations and dosages of recognised antibiotics and the use of genotyping in order to enhance eradication, novel antimicrobial agents are also being sought. Sulforaphane (found in broccoli) has *in-vitro* activity against *H. pylori* and a study *in-vivo* in nude mice showed that after treatment, in 8 out of 11 mice, *H. pylori* had been eradicated. Similarly, antimicrobial activity has been detected in plant and spice extracts and oils, for example carvacrol and nerol. An additional therapeutic approach is to add bovine lactoferrin to an established eradication regimen (rabeprazol...
+ clarithromycin + tinidazole). In a study of 150 patients, the eradication rate was 92% in the combination with bovine lactoferrin compared to 71% without. Probiotics might also be beneficial in the case of *H. pylori* infection. According to the studies of Cruchet *et al.*, regular ingestion of *Lactobacillus johnsonii* La1 may be an alternative to treat children infected with *H. pylori*.

### 3.2 Vaccine

Vaccine development is also an important target and although good results have been obtained in mouse models, this has thus far not translated into efficacy in humans. In one study in mice, the delivery of the *H. pylori* catalase gene in an attenuated *Salmonella typhimurium* showed protection in 61% of the mice. A different approach is to use immunomodulators. For example, the use of unmethylated CpG oligonucleotides (which are characteristic of bacterial DNA) enhances innate immunity, increasing chemokine production. In a mouse experimental system with an established *H. pylori* infection, the use of CpG oligonucleotides caused a reduction of the bacterial load in the stomach.

### 3.3 Adhesion at the epithelial surface and inhibitor development

The initial step of most infectious diseases is adhesion of the pathogenic organism to the host tissue. Once adherent, the organism is able to avoid removal by the host’s clearance mechanisms and establishes an infection. *H. pylori* causes a chronic mucosal infection when adherent to the mucosal epithelium of the stomach. The most studied *H. pylori* adhesin-receptor interaction, and the one thought to be the most important, is between the Blood group Antigen Binding Adhesin (BabA) and the Lewis b (Le b) antigen, the blood group antigen expressed by gastric epithelial cells. The study of Sheu *et al.* has confirmed this role: patients expressing Le b were shown to have a higher *H. pylori* density on the mucosal surface compared to Le b.
negative patients. However, in Le b negative patients, the results suggest that Lewis x (Le x) and Lewis a (Le a) may become the alternative major receptors for *H. pylori*. *H. pylori* has been shown to co-localise *in situ* with the gastric secretory mucin MUC5AC\(^{18}\) and expression of MUC5AC is associated with both Le a and Le b expression in the human stomach. It is now clear that the mucin MUC5AC is in fact the carrier of the Le b molecule in normal gastric tissues\(^{19}\).

It has also been shown that the expression of the Trefoil protein TFF1 mirrors the localisation of *H. pylori* in the gastrointestinal tract\(^{20}\). In the gastrointestinal epithelium, TFF proteins are expressed in a site-specific manner, TFF1 being primarily located within the foveolar pit cells of the gastric body and superficial regions of the antral glands\(^{21}\). Studies by Clyne *et al.*\(^{22}\) have shown that *H. pylori* is able to bind to TFF1, suggesting that alongside Le b, TFF1 may also be a receptor for *H. pylori in vivo* and may provide another reason for its tropism for gastric tissue.

Interestingly, it has previously been shown that upon infection with *H. pylori*, TFF1 expression is up-regulated in AGS cells and in T84 monolayers\(^{23}\). This TFF1 up-regulation has been shown to be CagA dependent. These findings therefore suggest that the ability of CagA+ strains to up-regulate TFF1 *in vivo* should confer on the organism an enhanced ability to colonise the gastric mucosa.

Because micro-organisms are becoming increasingly resistant to current anti-microbial agents, new strategies are constantly being sought to combat infection. One such strategy is to target this first step of microbial infection- adhesion, by using molecules that mimic the microbial adhesin or its complementary host cell receptor\(^{24}\). It has been shown that by pre-incubating *H. pylori* with soluble glycoproteins presenting the Le b antigen, the adhesion of *H. pylori* to stomach sections could be inhibited\(^{25,26}\). The sodium salt of 3’S-3’S-sialyllactose, a receptor analogue) was
shown to inhibit adhesion of *H. pylori* in vitro and in animal models of infection\(^{27,28}\). In recent clinical trials, however, 3’SL failed to suppress or cure *H. pylori* colonisation\(^{29}\). Another potential inhibitor has been investigated by Bai *et al.*\(^{30}\). An analogue of BabA, known as recombinant BabA (rBabA), was found to partially inhibit binding of *H. pylori* to gastric epithelial cells and significantly inhibit adhesion to the gastric cancer cell line MGC-803. If indeed TFF1 is a receptor for *H. pylori* in the human stomach, as suggested by Clyne *et al.*\(^{22}\), then their studies have also demonstrated a potential anti-adhesion therapy. They found that adhesion of *H. pylori* to TFF1-dimer coated beads was inhibited by either pre-incubating the bacteria with soluble recombinant TFF1 or by pre-incubating the beads with anti-TFF1 monoclonal antibody.

Plant extracts have also been studied as a source of *H. pylori* inhibitors. Successful inhibition of adhesion has been shown using cranberry juice\(^{31}\) and the seaweed *Cladosiphon fucoidan*\(^{32}\). Shibata *et al.*\(^{32}\) found that the seaweed inhibited adhesion of *H. pylori* to human gastric cells. Recently, they have shown that *C. fucoidan* inhibits adhesion of *H. pylori* to porcine gastric mucin and by adding the plant to the drinking water of infected Mongolian gerbils, the prevalence of animals with infection was shown to be markedly reduced\(^{33}\). Studies carried out by our group have shown that the spice turmeric is able to inhibit the adhesion of *H. pylori* to human stomach sections. By pre-incubating the bacteria with turmeric, we found that adhesion of *H. pylori* to both Lewis a and b stomach sections was inhibited by 55 and 60% respectively\(^{34}\). Lengsfeld *et al.*\(^{35}\), using the same method, have showed that by pre-incubating *H. pylori* with a fresh juice preparation of the fruit of the okra plant [*Abelmoschus esculentus* (L.) Moench], adhesion of *H. pylori* to human stomach sections was almost completely inhibited.
4. GENOMICS, PROTEOMICS AND HELICOBACTER PYLORI

Analysis of the genome and the proteome of organisms may be used to identify potential virulence factors, potential vaccine candidates and to apportion function to proteins in the organism under study, by analogy with proteins of known function in other organisms. The use of comparative genomics can also lead to a greater understanding of genome evolution and its interaction with ecology.

4.1 Genome evolution

The $H.\ pylori$ genome is about half the size of that of $Escherichia\ coli$ and consists of 1637 predicted coding sequences with 1594 predicted proteins. The organism has a large number of outer membrane proteins and putative adhesins testifying to the importance of adhesion in its biology. It also codes for about 25 restriction endonucleases and 27 methylases, underpinning the importance of genetic exchange to $H.\ pylori$. $H.\ pylori$ has relatively few regulatory proteins compared to organisms with similar sized genomes although some of the methylases may have regulatory functions. Additionally, there are large numbers of long sequences of the same nucleotide (homo-polymorphic tracts), which are in part the basis for the known macro- and micro- genetic diversity of the organism. Analysis of the genome has provided a further possible explanation of this characteristic, in part by the lack of a recognisable DNA mismatched repair system (MMR) and an incomplete Base Excision repair system (BER), although $H.\ pylori$ may have evolved as yet undetermined repair systems.

The whole genome sequence of $H.\ pylori$ has been incorporated into the BioCyc website (BioCyc.org), setting up a database of predicted biochemical pathways with visualization and query tools. This can be used to identify unique metabolic pathways
Genetic differences may have significance in relation to virulence. Such differences can be identified either by the process of subtractive hybridisation, a method that amplifies differences between genomes, or by micro-array technology (Figure 1). In this latter case, only sequences that are absent from the un-sequenced strain can be identified. In subtractive hybridisation, the genome sequence of one strain (called the “driver”) is known and endonuclease generated DNA fragments from this strain are mixed with fragments from the related strain (called the “tester”) and specific sequences of the “tester”, which self-anneal rather than anneal with the “driver” DNA, are amplified by PCR. Using these techniques and DNA from two separate isolates (strain 26695 and J99) of \textit{H. pylori}, it was shown that about 7\% of the sequences were unique to specific strains of \textit{H. pylori} and thus, as strain variation is linked with virulence, by implication, these unique sequences may be related to the virulence of specific strains\textsuperscript{38}.

\textbf{4.2 Host /Pathogen interaction from a genome perspective}

The investigation of the whole genome expression of \textit{H. pylori} under natural conditions may throw light on the host/pathogen interaction and aid understanding of \textit{H. pylori} pathogenesis of disease. Studies of global expression of the \textit{H. pylori} genome combine micro-array hybridisation with a cDNA-PCR technique called selective capture of transcribed sequences (SCOTS) (Figure 2). Preliminary investigation of \textit{in-vivo} expression of \textit{H. pylori} genes on contact with gastric epithelium had shown that in those strains, in which the gene iceA was expressed, there was more inflammation of the mucosa. In further studies, investigating the expression of genes across the whole \textit{H. pylori} genome on contact with gastric epithelium, many genes were expressed. Some of these genes have been provisionally
annotated or identified by sequencing, for example, BabA and alpA, but the majority of them are of unknown function. Further investigation of this host/pathogen interaction will yield important information on the bio-ecology of this mucosal-associated organism occupying a unique ecological niche\textsuperscript{39}.

Analysis of the genome of \textit{H. pylori} has further indicated that there are genes having homologues in other bacteria, which are involved in the invasion process, for example, InvA (40\% homologous with \textit{Salmonella typhi} InvA). Similar homologues have also been identified for other genes important in intracellular survival, for example, superoxide dismutase (SOD). The gene in \textit{H. pylori} has considerable sequence homology with the SOD of known intracellular pathogens, such as \textit{Coxiella} and \textit{Legionella}. These genome based investigations call into question the appreciation of \textit{H. pylori} as a strictly extracellular pathogen\textsuperscript{40}.

4.3 Vaccine

Of the large numbers of potential antigens that occur in any organism, a subset may be selected based on criteria such as immunogenicity or abundance (this approach has been called “reverse vaccinology”) in order to streamline vaccine development\textsuperscript{41}. Additionally, the combination of rapid 2D electrophoresis with MALDI-TOF mass spectrometry shortens the investigative time. \textit{In-silico} predictions of abundance can be made from codon usage, which correlates with protein expression. However, codon usage is not the sole criterion of expression as \textit{in-silico} prediction does not robustly correlate with proteomic data. Specific motifs defining outer membrane proteins can also be used as predictors of potential vaccine candidates but again the correlation is not exact.

However, investigation of both the transcriptome and proteome using a combination of these predictive techniques has identified antigens that are highly protective in
model systems and are potential vaccine candidates42. These antigens are outer membrane proteins and are unique to *H. pylori*. Of 15 initial potential candidate antigens identified, three of them (designated HP0231, HP0410 and HP1098) were purified by PCR amplification of the gene, cloning into *E. coli* and purified by cobalt affinity chromatography. One of the antigen genes (HP1098) did not clone effectively and was not studied further. Of the two other antigens, protection studies in mice showed they were as effective as recognised vaccine candidates such as urease. This study demonstrates that genome analysis and reverse vaccinology can lead to the discovery of effective vaccine candidates.

5. VIRULENCE FACTORS

5.1 The cytotoxin associated gene A

CagA has been reviewed by Hatakeyma43. CagA is part of the cagPAI, a 40Kb DNA fragment containing between 27 and 31 genes. Some of them code for a type IV secretion system, by which CagA is injected into host cells, where it is phosphorylated. Thereafter, CagA induces the humming bird phenotype, characterized by spreading and elongation of the cell shape, and a deregulation of cell growth. This last event is suggested to play a critical role in the development of gastric cancer. De Luca et al.44 have shown that, in association with HspB (a protein with homology with the family of the heat shock proteins and shown to increase the risk of gastric carcinoma), CagA increases the expression of cyclin D3, the phosphorylation of the tumour suppressor Rb, a substrate of the cdk-cyclin D complexes, and the expression of the transcription factor c-jun, leading to the increase in cell proliferation of AGS cells, probably caused by a deregulation of the G1/S checkpoint of the cell cycle. Another role of CagA has now been described. Using Madin-Darby canine kidney
(MDCK) cells, Amieva et al.\textsuperscript{45} have revealed that \textit{H. pylori} targets the cell junctions where CagA recruits the tight-junction scaffolding protein ZO-1 and the transmembrane protein junctional adhesion molecule (JAM) to sites of attachment. This recruitment is independent of the phosphorylation of EPIYA (single letter amino acid code) motifs, which are tyrosine phosphorylation sites on CagA. Like VacA, CagA also alters cell barrier function and in addition causes dysplastic alterations in epithelial cell morphology.

As mentioned, \textit{H. pylori} has been divided into Type I and Type II strains based upon the presence of cagA and the secretion of vacA. However, this division does not take into account the presence of the totality of the cagPAI and the presence of the EPIYA motifs: (1) If the type IV secretion system is not functional, due to deletion in the cagPAI, then CagA will not be injected into host cell; (2) The degree of phosphorylation of CagA might influence its virulence and (3) Other genes in the cagPAI are essential for the induction of IL-8 secretion\textsuperscript{46}. Although Type I strains have been associated with more severe diseases, these features cannot be ignored when looking at the association with disease. Nilsson \textit{et al.}\textsuperscript{47} took into account the whole cagPAI and analysed 66 \textit{H. pylori} isolates from patients with duodenal ulcer, gastric cancer and non ulcer dyspepsia. Using DNA microarrays, they found that 76%, 15% and 9% of the strains contain all, some or none of the cagPAI genes, respectively. Among the 15%, 3 were cagA negative. The ability to induce IL-8 production in AGS cells was correlated to the presence of the complete cagPAI. Moreover, the presence of an intact cagPAI was correlated with the development of more severe pathology.

\textbf{5.2 The vacuolating cytotoxin}

The vacuolating cytotoxin A gene (vacA) is present in nearly all strains of \textit{H. pylori}
but only half of them produce the toxin (VacA) as a mature protein of 95KDa. Forty
to sixty percent of VacA protein is associated with the outer membrane of *H. pylori*,
the rest being secreted. Most studies have looked at the effect of the secreted protein,
but little is known about VacA located on the bacterial surface. Ilver *et al.*48 have
shown for the first time that VacA on the bacterial surface is directly transferred to
host cells upon contact, which then induces vacuolation in AGS cells. This study
questions the role played by the non-secreted and secreted VacA *in vivo*. The
vacuolating cytotoxin is a powerful virulence factor as it can target several cellular
types, for example, gastric epithelial cells, macrophages, neutrophils and mast cells
and several cellular compartments, such as the cytoplasmic membrane, the late
endosomal and lysosomal compartments and mitochondria. In epithelial cells, most of
the effects of VacA are due to its ability to form anion-selective channels. One well
studied effect is the formation of vacuoles in diverse mammalian cell lines; the
consequences being a marked decrease of the proteolytic activity in the endocytic
pathway, perturbation of antigen presentation and cell death49,50. VacA also induces
apoptosis by a mitochondria-dependent mechanism, which is characterized by a
decrease in mitochondrial transmembrane potential and cytochrome c release51. Two
studies of the same authors have now confirmed that this mechanism is due to the
formation of anion-selective channels52,53. One of the two studies also suggests that
cytochrome c release might occur as a downstream consequence of a VacA-mediated
activity resulting in mitochondrial transmembrane potential reduction53. However, the
location of the channel is still undetermined, i.e. endosomal/lysosomal compartments
or mitochondria.

Previous studies have described two possible mechanisms used by *H. pylori* to escape
from phagocytic cells.: - The first mechanism is the inhibition of its uptake by human
neutrophils and monocytes. This inhibition was dependant on the presence of the type IV secretion system\textsuperscript{54}. - The second mechanism is the formation of megasomes in which type I \textit{H. pylori} can survive\textsuperscript{55}. Odenbreit \textit{et al.}\textsuperscript{56} found that the type IV secretion system and CagA did not play any role in the resistance to phagocytosis and killing by phagocytic cells. A more recent study by Zheng \textit{et al.}\textsuperscript{57} confirms that CagA is not implicated in the survival of type I strains in macrophages but that \textit{H. pylori} strains expressing VacA arrest megasome maturation in association with the retention of tryptophan aspartate-containing coat protein (TACO), which is a marker of the early phagosome. This last study has also shown that type I and type II strains induced similar levels of apoptosis in macrophages. Menaker \textit{et al.}\textsuperscript{58} went further by showing that apoptosis was due to an alteration of the mitochondrial pathway and that both CagA and VacA play role, this finding being in contradiction with the finding by Zheng \textit{et al.}\textsuperscript{57}.

The main new finding over the past year is the data on the inhibition of activation and proliferation of T cells by VacA. One study has demonstrated that VacA was able to inhibit the proliferation of activated Jurkat T cells and fresh human peripheral blood lymphocytes. In Jurkat T cells, inhibition of proliferation was due in part to the inhibition of the nuclear translocation of NF-AT, resulting in reduction of IL-2 secretion and IL-2 receptor expression. In fresh human peripheral blood lymphocytes, they showed a G1/S cell cycle arrest\textsuperscript{59}. This finding in Jurkat T cells has been confirmed by another study clearly showing that the inhibition of NF-AT translocation is due to the capacity of VacA to form ion channels in the cell membrane of T cells\textsuperscript{60}. Additionally, they have found that VacA activated p38 stress kinase and induced Rac-1-dependent cytoskeleton rearrangement; these two mechanisms are ion channel-independent. Both studies are discussed by Montecucco and de Bernard\textsuperscript{61}. A more
recent study has demonstrated that the inhibition of the proliferation of activated primary human CD4+ T cells by VacA is not dependent either on the inhibition of the secretion of IL-2 or the activation of NF-AT, rather the cells undergo anergy due to an arrest in the cell cycle. This last result needs further investigation but seems to confirm the finding by Gebert et al. These several studies underline the complexity of the inhibition of T cells by VacA and especially the type and state of T cells used.

6. GASTRIC CANCER

It is now recognised that *H. pylori* infection can lead to the development of gastric cancer (GC), but the exact mechanism by which this is brought about is still unclear. As not all persons infected with *H. pylori* develop gastric cancer, it is clear that there is more than one factor involved in disease outcome. Factors that increase the risk of gastric cancer development have been shown to include virulence of the infecting *H. pylori* strain, host genetic factors and diet (Table 1).

Past studies have shown that persons infected with *H. pylori* strains, which are cagA+ or have the vacA s1m1 genotype, are more likely to develop severe gastric diseases, in particular GC\(^{62,63}\). It has recently been shown that the presence of cagE in infecting strains may also incur a small risk of GC development\(^{64}\). Furthermore, Rad et al.\(^{65}\) have shown that the vacA s1 genotype is linked to a greater risk of developing GC, especially if in combination with other ‘high risk’ host markers such as the host IL-1\(\beta\) genotype IL-1\(\beta\) -511T/-31C and the genotype of its receptor IL-1RN*2. It has previously been proposed that the development of ulcer or adenocarcinoma is related to the presence of the triple positive genotype vacAs1 cagA and babA2\(^{66}\). More recently, several studies have confirmed that combinations of *H. pylori* virulence genes are associated with more severe gastric disease. Strains which co-express cagA
and babA2 have been linked to GC\textsuperscript{67,68}. The cagA/vacA s1 \textit{H. pylori} genotype was found to be most common in GC patients in the X’ian area of China\textsuperscript{69}. Zambon \textit{et al.}\textsuperscript{70} showed that patients are at a higher risk of developing intestinal metaplasia (IM; and hence GC) if infected with \textit{H. pylori} strains which co-express cagA, vacA s1 m1 and babA2, while the studies of Koehler \textit{et al.}\textsuperscript{71} found the frequency of \textit{H. pylori} strains with the combined vacA s1a/iceA1 genotype was highest in patients with adenocarcinoma.

Several potential new \textit{H. pylori} virulence genes have been identified and are thought to be linked to GC development. According to the study of Santos \textit{et al.}\textsuperscript{72}, a novel \textit{H. pylori} gene, \textit{JHP947}, may be implicated in the development of GC and duodenal ulcer. This study was carried out on 200 patients who were positive for \textit{H. pylori} infection and presented with either GC, duodenal ulcer or gastritis. They found that in patients with gastritis, only 44\% were colonised with \textit{JHP947}-positive strains, whereas \textit{JHP947}-positive strains were found in 79.2\% and 86.1\% of people with duodenal ulcer or GC, respectively. The presence of the \textit{JHP947} gene was also shown to be associated with the cagA+ genotype. Further studies are required to confirm the role of this potential new virulence marker in GC.

Three \textit{H. pylori} proteins have been identified that seem to be linked to the presence of GC\textsuperscript{73}. One of the proteins was identified as acylneuraminate cytidylyltransferase. The other two were novel proteins (having no identifiable matching homologue).

Considerable interest has focused on the relationship between disease and the combination of \textit{H. pylori} virulence factors, environmental and host factors, but little has been done to assess the role of colonisation pattern (and hence adhesion), suggested to be important in determining disease outcome\textsuperscript{74}. In the study by Akada \textit{et al.}\textsuperscript{75}, co-infection of mice with strains SS1 and X47 showed that the former strain was
four times more abundant in the antrum than X47 and that the reverse was true for X47; being more abundant in the fundus than the antrum. In order to study the binding of \textit{H. pylori} to different areas of the stomach, transgenic mice, that were deficient in parietal cells (found mainly in the fundus of the stomach), were infected with a strain of the organism that recognised a specific glycan receptor (NeuAc $\alpha$2,3 Gal$\beta$1,4)\textsuperscript{76}. In wild type mice, the organism bound to a region of the stomach at the junction between the antrum and fundus. However, in the transgenic animals, a further receptor was found, due to the genetic ablation of parietal cells, allowing the organism to bind in the fundus of the stomach. This study emphasizes the importance of glycan receptors and parietal cells in determining tissue tropism for \textit{H. pylori}, and the need to consider the evolution of pathology.

7. NON-PYLORI HELICOBACTER SPECIES

Since the discovery of \textit{H. pylori} and the establishment of the Genus \textit{Helicobacter}, there have been numerous additions to the Genus. \textit{Helicobacter} species can broadly be divided into two categories- those found exclusively in the stomach e.g. \textit{H. acinonychis} (cheetah), \textit{H. felis} (cat, dog) and those found principally in the lower intestine and hepatobiliary system e.g. \textit{H. hepaticus} (rodents), \textit{H. trogontum} (rodents). More recently \textit{Helicobacter} have been isolated from dolphins (\textit{H. cetorum}) and two new un-named \textit{Helicobacter} species from harp seals\textsuperscript{77}. The interest in the isolation of \textit{Helicobacter} species from a wide range of animals covers three important areas. Firstly, \textit{H. acinonychis} has been isolated from lions and tigers from geographically separate animal groups and investigated using random amplified polymorphic DNA fingerprinting (RAPD). Two main groups were identified and in all isolated strains the cagPAI was absent and the vacA gene degenerated. Between strains, there was a 2\%
amino acid substitution and an 8% difference compared to the homologues in *H. pylori*. Experimental co-infection with *H. pylori* in mice led to recombinant variants. Thus comparative analysis of closely related species has the potential for increasing the understanding of both host/pathogen interaction and genome evolution.

Secondly, as several of these animals can be used as model systems, they are important in understanding the pathogenesis of gastroduodenal disease caused by *H. pylori* and for the evaluation of vaccine development. The most common animal model that is used in vaccine assessment is colonisation of mice with *H. felis*. Colonisation of the human stomach with *H. pylori* can lead to gastric lymphoma, and similarly colonisation of the mouse with *H. felis* can also lead to lymphoma. Vaccination of mice with *H. felis* prior to infection not only can prevent colonisation by the strain but also protects against the development of lymphoma. This exciting development is the first indication that vaccination may be useful against malignancy.

Thirdly, as some of these animal *Helicobacters* (notably *H. hepaticus* and *H. bilis*) have been linked to the development of liver cancer or inflammatory bowel disease (IBD), they are of great interest in relation to what they can reveal about these conditions in humans. In particular, attention has focused on *H. hepaticus* and recently the whole genome has been sequenced. Additionally, some *Helicobacter* species (*H. cinaedi*, *H. fenellae*) are recognised pathogens in humans, giving rise to colitis, usually in homosexual men.

In a number of animal models of IBD, involving cytokine knockout mice, e.g. IL-10−/−, infection with *H. hepaticus* (and *H. bilis*) can give rise to inflammation of the colon. An important virulence factor involved in this inflammatory process is the cytolethal distending toxin (cdt), which causes cell cycle arrest in G2/M and subsequent cell enlargement, as transpositional mutagenesis of the cdt gene cluster
severely diminishes the capacity to induce colitis whist not affecting colonisation\textsuperscript{81}.

The induction of IBD by \textit{Helicobacter} species in animals has led to the search for similar organisms in humans with contradictory results, although overall there is no evidence that \textit{Helicobacter} species (or \textit{H. pylori}) are related to IBD. Most studies have used PCR. In our study of 72 patients (35 with IBD), \textit{Helicobacter} species were detected in only 6 subjects (3 IBD, 3 controls)\textsuperscript{82}. In another study, \textit{Helicobacter} species were not detected in any of the 50 patients or 25 controls\textsuperscript{83}, yet in a third study, 6 \textit{Helicobacter} species were detected in 43 patients and only once in 23 controls\textsuperscript{84}. A study using culture, detected only \textit{H. pylori} on the colonic mucosa but there was no correlation with disease\textsuperscript{85}. Most evidence to date would suggest there is no relationship between colonisation with species of \textit{Helicobacter} and the development of IBD in humans.

In addition to its role in animal models of IBD, \textit{H. hepaticus} can induce liver tumours in mice of certain genetic backgrounds. Again this has prompted a search for \textit{Helicobacter} species in patients with hepatobiliary problems. Using PCR, two studies have found \textit{Helicobacter} species DNA (principally \textit{H. pylori}) more commonly in patients with hepatitis C-related cirrhosis or hepato-cellular carcinoma\textsuperscript{86,87} whereas one study\textsuperscript{88} could not detect any \textit{Helicobacter} in liver samples. Similar contradictory results have been reported for the association between \textit{Helicobacter} species and cholelithiasis with one study demonstrating an association whilst another did not\textsuperscript{89,90}.

Finally, despite no definite evidence of \textit{Helicobacter} species being related to human malignancy or gall stone disease, there is a strong indication from serological studies that patients with autoimmune chronic liver disease have a higher prevalence of serum antibodies against non-gastric \textit{Helicobacter} species compared to a healthy control population\textsuperscript{91}. The significance of this finding remains to be determined.
8. CONCLUSION

Considerable advances have been made in understanding the evolution of the organism and pathogenesis of disease since the first isolation of *H. pylori*. The sequencing of two strains of *H. pylori* has provided a wealth of data that will be useful in understanding the pathogenesis of disease, microbial evolution and highlight potential therapeutic targets and potential vaccine candidates. It is this area of bioinformatics that, in the future, is likely to advance understanding of disease progression and aid in the development of novel therapies. One particular aspect of these genomic studies that is arousing considerable interest, and will certainly be followed up in future research, is the identification of host and genome polymorphisms and how these interact in relation to the development of severe disease. A second area of research that is becoming increasingly important is the development of new therapies, which is driven by the emergence of antibiotic resistance. The search for new therapies will be based not only on the genomic studies mentioned above, but on a search for novel sources of antimicrobial agents and investigations into modulation of the immune system. Finally, because of the importance of both IBD and liver cancer, it is reasonable to suppose that more studies will be undertaken on the role of the ever expanding Genus *Helicobacter* in relation to human disease.
REFERENCES


polymorphism influences the effect of cytochrome P 2C19 genotype on the cure rate of 1-week triple therapy for Helicobacter pylori infection. Am. J. Gastroenterol. 98, 2403-2408.


52. Willhite, D.C., Cover, T.L., Blanke, S.R. (2003) Cellular vacuolation and mitochondrial cytochrome c release are independent outcomes of *Helicobacter pylori*
vacuolating cytotoxin activity that are each dependent on membrane channel formation. *J. Biol. Chem.* **278**, 48204-48209.


### Table 1  Factors involved in determining disease outcome in patients with H. pylori infection

<table>
<thead>
<tr>
<th>Factor</th>
<th>Risk of Disease</th>
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<tbody>
<tr>
<td><strong>Host Factors</strong></td>
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<tr>
<td>IL-1ß genotype (High production)</td>
<td>Gastric cancer</td>
</tr>
<tr>
<td>HLA type</td>
<td>Possible role in gastric cancer</td>
</tr>
<tr>
<td>Mucin type (small alleles at MUC1+6 loci)</td>
<td>Gastric Cancer</td>
</tr>
<tr>
<td><strong>Environmental Factors</strong></td>
<td></td>
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<tr>
<td>Helminth infection</td>
<td>Reduced cancer risk</td>
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<tr>
<td>Smoking</td>
<td>Gastric Cancer</td>
</tr>
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<td>Poor Childhood environment / living conditions</td>
<td>Increase risk of H. pylori colonisation</td>
</tr>
<tr>
<td><strong>Microbial Factors</strong></td>
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<td>Colonisation patterns:</td>
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<tr>
<td>Antrum</td>
<td>Duodenal ulcer disease</td>
</tr>
<tr>
<td>Fundus</td>
<td>Gastric ulcer, Cancer</td>
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<td>Virulence Genes:</td>
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<tr>
<td>vacA</td>
<td>Gastric Cancer / peptic ulcer disease</td>
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<td>vacA (s1a/m1)</td>
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<td>vacA (s1b/m2)</td>
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<td>iceA1</td>
<td>Peptic ulcer disease</td>
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<td>ptpA</td>
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</tr>
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<td>Adhesins:</td>
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<tr>
<td>BabA2</td>
<td>Higher risk of disease</td>
</tr>
</tbody>
</table>
Figure 1

Schematic of Subtractive Hybridization

Figure 2

Schematic of Selective Capture Of Transcribed Sequences (SCOTS)