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An Update on *Helicobacter Pylori* Microbiology and Infection for the New Millennium

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The finding of the bacterium *Helicobacter pylori* in patients with symptomatic gastric diseases was a breakthrough for both treatment of peptic ulcer disease and studies of other infectious diseases. *Helicobacter pylori* infection is rare among the young, indicating that improved childhood living conditions have halted the transmission of the bacterium within families, with a parallel decrease in symptomatic gastroduodenal diseases. Extensive strain variation in *H. pylori* has been demonstrated at both the genomic and the protein level, and the interstrain variation is higher than in any other bacterium studied so far. Pathogenic markers in *H. pylori* and host genetics are both of importance for disease outcome. Genotypic or phenotypic markers of *H. pylori* strains may be used to discriminate patients who should undergo eradication therapy from those who might not benefit from it. Possible positive effects of the infection are still under investigation, and several hypotheses regarding the etiology of diseases in different parts of the stomach have been proposed. To be able to separate the disease-causing infections from the silent infections is a real challenge for the new millennium, and one of the most important issues for therapy and prevention, in the research field of *H. pylori*.

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REVIEW

The genus Helicobacter and its associated diseases

Spiral-shaped bacteria have been observed in studies including human patients since 1889, but the organism *Helicobacter pylori* was discovered almost 100 y later (1). In humans, *H. pylori* is the major cause of peptic ulcer disease, and the finding of a colonizing bacterium in the gastric mucosa was a breakthrough in the studies of this common complaint. Before the discovery of the bacterium, studies had been focusing on gastritis as the cause of peptic ulcer. In 1975, histopathological studies by Steer indicated the interaction of a bacterium with gastritis, and the research started to involve microbiology for the isolation of this new bacterium and its possible interactions with a variety of gastric diseases. The isolation and culture of *H. pylori* from patients with abdominal complaints was not successful until 1982 when Marshall and Warren identified *Helicobacter* (formerly *Campylobacter*) *pylori* in human gastritis (2). Since then, a tremendous number of studies has been performed on *H. pylori* and its involvement in both gastric and extragastric diseases, and the search for useful therapies to eradicate the bacterium has been extensive. Combinations of proton pump inhibitors (PPIs), such as omeprazole and lansoprazole, H₂-blockers, bismuth compounds and combinations of different antibiotics are now in use as eradication therapies, and the use of these in peptic ulcer disease has significantly reduced the number of relapsing ulcers. The evidence for *H. pylori* as a causative agent for functional, non-ulcer, dyspepsia is still equivocal but some consensus reports state that eradication treatment is advisable in these patients (3). However, dyspepsia is a heterogeneous condition, and it is likely that several pathophysiological disturbances contribute to the complex origin of symptoms.

When observed in vivo, *H. pylori* is a spiral-shaped or curved rod, a few micrometers long and actively motile (4, 5). Organisms of *H. pylori* can also be found in a horse-shoe-like U-form and a round, or coccoid, form in older cultures. *Helicobacter pylori* have 4–6 unipolar sheathed flagella, which are of importance for bacterial motility. In the stomach, most of the *H. pylori* organisms are found in an extracellular location in the gastric mucus and a few organisms are found adhered to the mucosa. Electron micrograph pictures revealed an intracellular location of the bacterium, and the uptake of *H. pylori* into human epithelial cells has been shown by time-lapse photography (6, 7).

Adherence and entry of *H. pylori* into AGS cells has been studied in vitro (8). An intracellular location of *H. pylori*, both in cells of the gastric mucosa and in monocytes and macrophages, has been proposed as a mechanism for the bacterium by which it avoids the immune system and antibacterial treatment (9).

Some features are shared among almost all species included in the genus *Helicobacter*, such as the presence of sheathed flagella, a guanine and cytosine (G + C) content of chromosomal DNA of 35–44 mol%, and strong urease activity (5). Several intestinal *Helicobacter* species such as *H. canis* and *H. cinaedi* are urease negative. The bacteria are highly diverse in cell morphology with respect to cell length, number and location of the flagella. The genera most closely related to *Helicobacter* are *Wolinella* and *Campylobacter*, as determined by 16S rRNA sequencing. Many different animal species are infected by their own *Helicobacter* species other than the human pathogen *H. pylori*, which suggests a common ancestor for organisms colonizing the gastric mucosa, intestines and liver (10). In addition to gastric diseases, some *Helicobacter* species may

cause colitis, hepatitis, hepatic adenocarcinoma or hepatic adenoma in their hosts. Infection by *H. pylori* has been experimentally induced in several animal species, including, pigs, dogs, cats, macaques, gerbils and mice. Chronic gastritis appeared after an incubation period in all animal species tested so far. Gastric *Helicobacters* other than *H. pylori* have been used to infect animals experimentally. Ferrets were infected with *H. mustelae*, and mice, rats and dogs were infected with *H. felis*. Chronic gastritis, gastric ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma were induced in these animal models.

Helicobacter pylori infection, together with other host genetic, environmental and bacterial virulence factors, may induce chronic gastritis in certain susceptible individuals. Chronic active gastritis may progress to gastric atrophy with intestinal metaplasia, and *H. pylori* is responsible for this change (11, 12). By causing inflammation and cell proliferation, *H. pylori* enhances the likelihood of mutation in gastric epithelial cells. The mutations are accumulated during a latency period before gastric cancer appears, which is then more likely to develop if the lifelong infection is acquired early in childhood. In some persons *H. pylori* infection stimulates an increased gastric acid secretion which may lead to duodenal ulcer disease, and in others the infection leads to a reduced acid secretion which predisposes for cancer development. Oncogenic factors other than *H. pylori* may then be involved in the progression to dysplasia and gastric cancer, and *H. pylori* infection is also a strong predictor of atrophic gastritis. Since the observation that *H. pylori* was associated with a variety of gastric diseases, interest has also been focused on the possible protective role of *H. pylori* in gastroesophageal reflux disease and in cancer of the esophagus. The role of *H. pylori* in gastric cancer development is also still under debate (13–16). The bacterium *H. pylori* is a predisposing condition for both the development of gastric adenocarcinoma and gastric B-cell MALT lymphoma, but not for adenocarcinoma arising in Barrett's esophagus. Based on the association between *H. pylori* and gastric cancer, the decision was made by IARC in 1994 to name *H. pylori* as a human carcinogen. Several meta-analyses have showed that *H. pylori* infection is a risk factor for gastric cancer by an overall odds ratio (OR) of 1.92 [95% confidence interval (CI) 1.32–2.78], a pooled OR of 2.04 (95% CI 1.69–2.45) and a risk ratio (95% CI) for gastric cancer of 2.5 (1.9–3.4) (17–19). Some epidemiological studies have failed to show an association between *H. pylori* and gastric cancer, and the evidence for a causal relationship has been weakened as more studies have been published (20).

Etiological factors in the host and in the environment other than *H. pylori* must be considered in future epidemiological studies, as well as the fact that different strain types of *H. pylori* are observed to be associated with different gastric cancer risks (14). There is now evidence

Table I. Model of increased likelihood of different diseases from *Helicobacter pylori* infection (21, 22)

Host parietal cell mass/function (acid secretion)	Virulence of infecting <i>H. pylori</i> strain	
	High (<i>cagA</i> +)	Low (<i>cagA</i> -)
Higher than normal	Duodenal ulcer	Duodenal ulcer
Normal	Duodenal ulcer	Gastritis
Lower than normal	Gastric cancer	Gastritis

that *H. pylori* has the capacity to induce intestinal type gastric adenocarcinoma in the antrum in vivo, at least in an animal model using Mongolian gerbils (21). A multifactorial hypothesis of gastroduodenal disease development was proposed, where *H. pylori* is only 1 of many etiological factors involved in disease progression (22). A model for gastric cancer and duodenal ulcer development has been suggested, taking age at acquisition of the infection, gastric acid output and strain type of *H. pylori* into account (Table I) (23, 24). The specific effect of *H. pylori* infection in different persons might be explained by differences in bacterial strains, host genetics, dietary factors or environmental factors. A model for the relationship between age at acquisition of *H. pylori* infection and risk of adverse clinical consequences has also been proposed (Table II) (25). A model that explains how the gastric inflammatory response, triggered by *H. pylori* and cytokines induced during infection, contributes to the pathogenesis of cancer was also proposed (26). *Helicobacter pylori* infection increases epithelial cell turnover, cell proliferation and apoptosis, and the bacteria cause DNA damage by free radicals, oxidants and reactive nitrogen species (27–30). An etiological role of nitrate-reducing bacteria inhabiting the gastric mucosa in gastric cancer patients has also been suggested (31).

Epidemiology, transmission and detection of Helicobacter pylori infection

The epidemiology of *H. pylori* infection is of great importance for the understanding of this common worldwide infection. The prevalence of *H. pylori* infection in the world is assumed to be as high as 50%, with a higher prevalence in developing than in developed countries. The prevalence varies substantially depending on the economic development of each individual country (32). The rate of new acquisitions, the incidence rate, of *H. pylori* infection is

Table II. Relationship between age at acquisition and *Helicobacter pylori*-induced disease (23)

Acquisition age	Consequence
Youngest (childhood)	Distal gastric cancer (non-cardia)
↓	Corpus gastric ulcers
Oldest	Prepyloric gastric ulcers and duodenal ulcers
Never	Esophageal disease

approximately 3% per decade for adults in developed countries, which is far below the acquisition rate for children. In developing countries, most children become infected during the first 5 y of life, resulting in a high prevalence of *H. pylori* in adults (33). Long-term residents in developing countries have a higher risk of acquiring *H. pylori* infection than if they lived in industrialized nations (34). A decline in *H. pylori* prevalence has been observed in children in both developed and developing countries, and the current acquisition rate during childhood in industrialized countries appears to be very low. Familial clustering of *H. pylori* infection was found, and the risk of infection in children was increased in families with low social status (35). Spontaneous clearance and transient infections in children seem to be common, and the reinfection rate in children is low (36, 37). Seroconversion in adults is rare, but elimination of infection occurs in some people without previous eradication therapy. Alcohol intake, especially in the form of wine, may reduce the odds of an active *H. pylori* infection among adults (38). In a Japanese study the seroreversion rate per year was calculated as 1.8% for children and 1.5% for adults (39). As the acquisition of *H. pylori* appears in childhood and follows the person as a chronic infection throughout life, a lower seroprevalence of *H. pylori* in older age groups is expected in the future. This phenomenon is called a birth-cohort effect, and it is responsible for the decrease in occurrence of *H. pylori* infection in cohorts of people born more recently in the twentieth century.

As with other bacterial and viral infections, a mix of environmental and genetic factors contributes to both acquisition of and susceptibility to *H. pylori* infection, which then determines the outcome of clinical disease (40, 41). Low levels of education and income, poor housing and sanitary conditions, large family size, sharing a bed in childhood and household crowding were closely associated with *H. pylori* infection. An increased risk of acquiring the infection at work has also been studied (42). Among the genetic factors of importance for an increased risk of *H. pylori* infection is male gender, blood group A and O, and Lewis b blood group antigen. Distribution of human leukocyte antigen (HLA)-DQA1 alleles differs between *H. pylori*-positive and -negative persons, indicating that HLA gene polymorphisms contribute to either susceptibility or resistance to *H. pylori* infection (43). This finding was not confirmed in a study using randomly selected healthy volunteers (44). Host susceptibility to the colonization of different *H. pylori* strains was shown in animal models, and it was found that certain strains of *H. pylori* are better suited for long-term survival in different hosts (45, 46).

Two major pathways for transmission of *H. pylori* are suggested: oral–oral transmission, which most often occurs in childhood within families, and a possible fecal–oral route that may occur particularly in developing countries (33). Multiple pathways must be considered, since *H. pylori* organisms were isolated from a number of locations includ-

ing feces, saliva, dental plaque and the domestic cat. Zoonotic transmission of *H. pylori* to humans and animal-to-animal cycling of the organism may also be of importance (33). Transmission from spouse to spouse via the oral–oral route or by a common source of infection is also possible, since the risk of becoming infected as an adult increases with the time lived with an *H. pylori*-infected partner (47). Both invasive and non-invasive diagnostic test methods are available for the detection of *H. pylori* infection in humans, and the most common methods are presented in Table III. For specific detection of *H. pylori* organisms in environmental samples, feces, gastric juice and biopsies, and for detection of *H. pylori* genes, polymerase chain reaction (PCR) techniques are used. The genes used for PCR techniques to distinguish between *H. pylori* and other microorganisms include the adhesin encoding gene *hap*, the urease encoding gene *ureA* and the 16S ribosomal RNA gene (48).

Helicobacter pylori virulence factors and pathogenic mechanisms

Several bacterial factors have been suggested to be responsible for a successful infection by *H. pylori*: high urease production which increases the pH and activates the host immune defense, flagella which facilitate the movement within the mucus layer, and adherence to gastric epithelium by different bacterial adhesins using hemagglutinins, laminin and Lewis b antigens as receptors. Persistence of the infection occurs when *H. pylori* escapes the immune defense and suppresses the host's cellular immune response by the action of *H. pylori* catalase, superoxide dismutase or urease (49, 50). *Helicobacter pylori* presents tissue tropism, binding only to surface mucous cells in the gastric epithelium. Attachment of *H. pylori* to the epithelium alters disease outcome by interacting with the cellular and humoral immune response and by inducing parietal cell autoantibodies (51). *Helicobacter pylori* may also produce biofilms of water-insoluble products, which may facilitate its survival and growth and enhance resistance to the host's defense (52).

The blood group antigen Lewis b, when expressed on gastric tissue, works as a receptor for *H. pylori*. Lewis b expression, and subsequent binding of *H. pylori* to gastric tissue, is lower in children and adolescents than in adults (53). The blood-group antigen binding adhesin (BabA), which binds to Lewis b, has also been identified (54). *Helicobacter pylori* lipopolysaccharides (LPS) express Lewis x and Lewis y blood group structures similar to those expressed on gastric human epithelium, which indicates that autoimmune reactions may be of importance for the severity of *H. pylori* infections (55). Single colonies of *H. pylori* isolated from a single host may present with a diversity of Lewis blood group structures, probably to obtain optimally host-adapted populations of bacteria (56). Host factors and bacterial factors both contribute to the mucosal damage.

Table III. Diagnostic test methods for detection of *Helicobacter pylori* infection

Test method	Test material	Detection result
<i>Non-invasive tests</i>		
¹³ C-urea breath test (UBT)	Respiratory CO ₂	Radiolabeled CO ₂
Immuno-chromatography (rapid tests)	Whole blood, serum	Anti-H. pylori IgG, IgM and IgA
Enzyme immunoassay (EIA)	Serum, stool	Anti-H. pylori IgG, IgM and IgA
Enzyme-linked immunosorbent assay (ELISA)	Serum, saliva	Anti-H. pylori IgG, IgM and IgA, anti-CagA IgG
Western blot, immunoblot	Serum	IgG response towards specific H. pylori antigens such as CagA and VacA
Complement fixation	Serum	Anti-H. pylori IgG and IgA
Latex agglutination/haema agglutination assays	Serum	Anti-H. pylori IgG, IgM and IgA
<i>Invasive tests (require endoscopy)</i>		
Rapid urease test (RUT) (CLO-test)	Biopsy	Gastric urease activity
Direct Gram stain	Biopsy	H. pylori-like organisms (if Gram-negative spiral organisms observed)
Culture, non-selective or selective	Biopsy	H. pylori (if Gram-negative, catalase-positive, oxidase-positive, urease-positive)
Histology	Biopsy	H. pylori-like organisms [by hematoxylin & eosin (HE) Warthin Starry silver stain, modified Giemsa stain or acridine-orange stain]
Immunohistochemistry	Biopsy	H. pylori (by immunoperoxidase or immunofluorescent techniques using anti-H. pylori monoclonal or polyclonal antibodies)
<i>PCR techniques</i>		
Different assays for different genes	Various sources	H. pylori DNA from cultured organisms, biopsy specimens, gastric juice, dental plaque or feces

When persistence of *H. pylori* infection is established, several virulence factors are associated with the outcome of clinical disease (Table IV) (49, 50, 57). The vacuolating toxin, VacA, which produces intracellular vacuoles in epithelial cells, and the CagA protein have both been used as markers for strains with an enhanced virulence. Immunoblotting can be used to find subjects harboring pathogenic strains of *H. pylori* by detection of specific antibodies towards proteins such as CagA and VacA. The presence of a pathogenicity island, the *cag* PAI, is also associated with increased pathogenicity of the infecting strain. Many of the genes residing in the PAI, including *cagA*, *picA* and *picB*, are now under investigation (58). Several of these virulence factors could possibly be used as indicators for treatment of infection, since strains expressing these factors are known to cause symptomatic disease in some people.

Helicobacter pylori genome

The high level of macrodiversity observed in the *H. pylori* genome is due to gene rearrangements on the chromosome and to variability in gene order. It has been discussed whether the genomic diversity in *H. pylori* may account for the chronicity of infection and whether multiple strain infections may expose the hosts to a variety of *H. pylori* strains with different abilities to induce disease (59). A selection of a recombinant *H. pylori* genotype with better growth was shown to occur during multiple strain infection in an infected patient (60). When the complete genome sequence of *H. pylori* was published by Tomb et al. in 1997, it was reported that *H. pylori* harbors outer mem-

brane proteins (OMPs) and dinucleotide repeats in coding sequences which are used for antigenic variation and adaptive evolution. This further explains the diversity observed among isolated strains (61). The genome of *H. pylori* (1700 kbp) is only about one-third the size of the *Escherichia coli* genome (4600 kbp) and contains only about one-tenth of the number of *E. coli* regulatory sequences, indicating that many genes involved in the adaptation to different environments are missing. This supports the notion that *H. pylori*'s only niche for colonization is the human stomach (62–64). More genome sequences from other prokaryotes have now been published or are forthcoming, and hopefully will speed up the understanding of the mechanisms by which pathogenic bacteria cause human disease. The genome sequence of the food-borne pathogen *Campylobacter jejuni* has been released, and comparisons with the *H. pylori* genome showed that 17% of the genes were specific to *H. pylori*, although the *C. jejuni* genome is of the same size and the 2 organisms are closely related (65).

By comparison of 3 *H. pylori* genes in strains from different populations, a high frequency of horizontal genetic exchange and recombination was shown compared with other microorganisms, but *H. pylori* seems to be clonal over short periods (66). By sequence comparisons between 2 unrelated isolates of *H. pylori* it was found that 6–7% of the genes were clustered in hypervariable regions and were specific to each strain (67). The overall genomic and allelic diversity, gene order and predicted sets of proteins encoded (proteomes) were more similar in the 2

isolates than predicted by earlier studies and by the notion that only certain strains of *H. pylori* in certain hosts cause severe disease. When *H. pylori* adapts to changes in its environment, the bacterium uses several methods to change its genotype: by point mutations predominantly in non-coding regions of conserved genes, by genomic rearrangements, and by horizontal gene transfer due to recombination in vivo with other strains, which can be observed as mosaic gene structures in *H. pylori* isolates (68). The bacterium may also alter its phenotype by changing the antigen expression and secretion of proinflammatory molecules. The *H. pylori* populations within a host may fluctuate, and multiple strain infections or infections with subtypes or clusters of closely related organisms of a *H. pylori* strain occur. Over time, small changes such as point mutations lead to variations within a strain and can then be detected as subtypes or clones of the same strain (genetic drift). Larger changes in the genome such as recombination events can lead to formation of 'new' strains of *H. pylori*, (genetic shift). Both genetic drift and genetic shift are assumed to appear in populations of *H. pylori* organisms, contributing to the genetic heterogeneity of *H. pylori*. By inducing mutations in existing genes, pathogenic organisms such as *H. pylori*, *E. coli* and *Hemophilus influenzae* may adapt to their novel environment and improve their fitness and chances of long-term colonization and survival within the host (69).

Pathogenicity islands (PAIs), regions of genes that codes for toxins, adhesins, invasins and other virulence factors, are found in a number of pathogenic bacteria including uropathogenic and enteropathogenic *E. coli*, *Vibrio cholera*, *Yersinia enterocolitica* and *H. pylori* (70). A part of the *H. pylori* genome, called the *cag* pathogenicity island

(*cag* PAI), has been extensively studied (58). The genes in the *cag* PAI are believed to code for more than 40 putative proteins, among them are genes similar to those from other bacterial species which are associated with flagellum assembly, proteases, translocases, sensors and permeases, and proteins involved in a secretion system for the export of virulence determinants involved in host-bacterial interactions. Some proteins are involved in interleukin-8 (IL-8) induction from the gastric epithelium and in pedestal formation.

A classification of strains into 3 types has been proposed: type I strains which are associated with severe disease pathology, intermediate strains, and type II strains which are attenuated in virulence (Table V). Type I strains express the VacA protein and contain not only the *cagA* gene, but the whole *cag* PAI (58). Type II strains do not express VacA and have no *cag* PAI. Intermediate strains do not have a fully intact *cag* PAI, and the strains express either the CagA or the VacA protein. In a study comparing type I and type II strains, the adherence to gastric cells by type I strains, but not by type II strains, was shown to be dependent on the upregulation of host cell receptors triggered by the bacterium (71). It was also suggested that increased adherence to Lewis b by type I strains, compared with type II strains, is dependent on protein synthesis from genes in the *cag* PAI.

Important Helicobacter pylori genes

The 2 genes studied most intensively over the years are the *vacA* gene encoding the vacuolating toxin, and the *cagA* gene encoding a cytotoxin-associated protein (57, 72). The *vacA* gene varies in its signal sequence and midregion, and the genotype influences levels of cytotoxin production. The

Table IV. *Helicobacter pylori* virulence (47, 48, 55)

Bacterial properties	Virulence factors	Genes/proteins involved
Colonization	Urease synthesis to increase pH	UreA, UreB, UreE, UreF, UreG, UreH, UreI, NixA, Hpn, HspA
	Motility by flagellar movements	Flagellins FlaA, FlaB, <i>flgE</i> , <i>flbA</i>
	Factors modifying acid secretion	LPS
Multiplication	Bacterial adhesins and cellular receptors	BabA and Lewis b, fimbriillar adhesin HpaA
	Iron capture system	Ferritin Pfr
Persistence	Protection against phagocytosis	Catalase, superoxide dismutase (urease activity), ammonia
	Disturbance of the local immune response	Urease, heat shock proteins HspA and HspB, catalase, ferritin
Damage to the gastric mucosa	Lesions of the mucosa	(Urease activity) ammonia, VacA, LPS, Lewis x and Lewis y, enteropathogenic-type tight adhesion, loss of mucosa integrity and enzymatic activities
Inflammatory reaction	Chronic inflammation	IL-8 induction by bacterial products expressed from genes in the <i>cag</i> PAI, neutrophil activation
Regulation of acid secretion	Acid hypersecretion	Gastrin hyperproduction (G-cells), reduced somatostatin production (D-cells)

Table V. The *cag* PAI: *Helicobacter pylori* type I, intermediate and type II strains (56)

Strain type	<i>cag</i> PAI	<i>cag</i> I, <i>cag</i> II, IS605	<i>cagA</i> /VacA	Virulence
Type I	<i>cag</i> intact	<i>cag</i> I fused to <i>cag</i> II, no copies of IS605	<i>cagA</i> +/VacA+	High
Intermediate	<i>cag</i> inverted, <i>cag</i> interrupted, <i>cag</i> with intervening sequence, <i>cag</i> partially deleted	<i>cag</i> I and <i>cag</i> II may be partially or totally deleted, >1 copy of IS605	<i>cagA</i> +/VacA- or <i>cagA</i> -/VacA+	Medium
Type II	<i>cag</i> totally deleted	No <i>cag</i> I or <i>cag</i> II, no IS605	<i>cagA</i> -/VacA-	Low

signal sequence of *vacA* has been shown to be closely related to the occurrence of peptic ulceration and atrophic gastritis. Differences observed in cytotoxin production between different strains of *H. pylori* cannot be explained only by signal and midregion sequences. Transcriptional differences seem to be responsible for the levels of cytotoxin produced (73), and the antibody response in the host is dependent on the *vacA* allele combination that is present in the infecting strain (74). Polymorphisms of the *vacA* gene seem to be associated with certain diseases in strains isolated from small geographic regions (75–77). The geographic distribution of *H. pylori* strains with different *vacA* alleles was shown when *H. pylori* strains isolated from 24 countries in Europe were compared (78). Distinct *H. pylori* lineages have also been found by comparisons of the *cagA* and *vacA* subtypes in strains collected from 9 different countries (79).

Approximately 50% of the *H. pylori* strains produce the vacuolating toxin and 60–70% of the strains are positive for the *cagA* gene. Most *cagA*-positive strains also express the CagA protein. The presence of the *cagA* gene in some, more pathogenic, *H. pylori* strains was associated with peptic ulceration and gastric adenocarcinoma (57). The *cagA* gene is used as a marker for presence of the *cag* pathogenicity island, where the *cagA* gene is encoded. Expression of the *cagA* gene is not necessary for the production of the vacuolating toxin, even though the 2 proteins are often found in and expressed by the same isolates. When Mongolian gerbils were challenged with *cagA*-negative or *cagA*-positive *H. pylori* strains, the *cagA*-positive strains gave a higher number of infected animals than the *cagA*-negative isolates (80). Strains positive for the *cagA* gene are associated with an increased gastric cell proliferation, but not with apoptosis (81). When comparisons of asymptomatic subjects and duodenal ulcer patients were performed, *cagA*-positive strains were associated with duodenitis and a higher bacterial density in the duodenal bulb than *cagA*-negative strains (82). The *cagA* gene alone cannot be used as a marker for increased severity of gastroduodenal disease, but a combination of the *vacA* s1 allele, the *cagA* gene and a gene called *iceA* (induced by contact with epithelium, alleles *iceA1* and *iceA2*) could be used to find strains which are more likely to cause ulcers in their hosts (83, 84). No association between these 3 genes and disease outcome was found when comparisons of strains from 4 different countries were performed (85).

Other interesting genes are *picA* and *picB*. The *picB* gene product, and not the CagA protein as first suggested, is responsible for IL-8 induction in the gastric epithelium in *H. pylori* infections (86).

By immunoblotting techniques, antibodies towards specific *H. pylori* antigens such as the virulence markers CagA and VacA can be revealed. This is therefore a useful method to detect infections with more virulent strains of *H. pylori* (87, 88). Many studies on the importance of antibodies to the CagA and VacA proteins in symptomatic diseases, such as active gastritis, peptic ulcer and gastric cancer, have been performed. Antibodies towards the *H. pylori*-produced CagA protein have been found in several studies to be associated with an increased risk of developing gastric cancer, especially the intestinal type of gastric cancer in the distal part of the stomach (89). CagA-negative *H. pylori* infections have been found to be only weakly associated with gastric malignancy. The effect of CagA-positive strains on the development of atrophic gastritis has also been extensively studied, and an association between the development of atrophic gastritis and infection by CagA-positive strains was found (90, 91). The development of intestinal metaplasia is also more common in subjects infected by CagA-positive *H. pylori* strains. In a multicenter study in 13 countries, the proportion of CagA-positive strains of *H. pylori* varied between the centers, but an increase in pepsinogen A and C levels and a decrease in pepsinogen A/C ratio were found in subjects infected with CagA-positive strains in all centers (92).

Various methods have been used for comparisons both within and between infected people to differentiate between clinical isolates of *H. pylori* (72). The same methods can be used in epidemiological studies, in studies of multiple strain infections and in studies of isolates before and after eradication therapy. The interhost diversity of isolated *H. pylori* strains has been shown to be remarkably high, although intrahost variation is low and multiple strain infections seem to be rare (93–95). Associations between specific fingerprinting patterns and disease outcome have been noticed in some, but not in all, studies (84, 96). Molecular relationships between *H. pylori* strains have been observed by genetic analysis of strains isolated from families, highlighting the importance of the genetic makeup of the infecting strain of *H. pylori* to result in symptomatic disease (97). PCR assays have been used to detect *H. pylori* organisms, macrolide resistance and the *cagA* gene directly in gastric juice and in gastric biopsy specimens (98, 99).

Treatment and antimicrobial resistance

Treatment of *H. pylori* infection is strongly recommended in patients suffering from peptic ulcer disease and MALT lymphoma. The Maastricht consensus report concluded that it is advisable to treat the infection in patients with functional dyspepsia and in those with a family history of gastric cancer, but it is not advised as prevention of gastric cancer in otherwise healthy, asymptomatic subjects (3, 100). Currently, there are no definitive data to show that eradication of *H. pylori* infection will lead to a sustained improvement of symptoms in functional dyspepsia patients. The most widely studied treatment regimen for the elimination of *H. pylori* is triple therapy during 1 week with a combination of 2 antibiotics (clarithromycin or amoxicillin and metronidazole) and a PPI (omeprazole, lansoprazole). H₂-Receptor antagonists may replace PPIs but this alternative is not commonly used. If the triple therapy fails, quadruple therapy based on PPI plus classic bismuth-based triple therapy (bismuth, metronidazole and tetracycline) can be used. Treatment failures of *H. pylori* may be due to low patient compliance, antimicrobial resistance, poor intracellular effect (e.g. amoxicillin) or failure of drug delivery across the gastric mucosa (101). The addition of PPIs to the therapy of *H. pylori* infection improves ulcer healing, increases eradication rates and decreases primary resistance, since the antibiotics are more effective when the intragastric pH is raised (102, 103). For the future, new combinations of 'old' antimicrobial agents, or new antimicrobial agents may be used for therapy of *H. pylori* infections (104, 105). Longer acting antibiotics and acid suppressants may reduce the length of therapy and increase patient compliance. Single capsules containing combinations of drugs for triple therapy, the linking of 2 drugs into 1, or the use of 'designer drugs' based on new knowledge about the *H. pylori* genome and the organism's life may also improve treatment. Designer drugs that inhibit the action of specific proteins essential for the survival of *H. pylori* in the gastric mucosa or more effective acid suppressants may also become available.

Differences in antibiotic resistance have been observed between clones of genotypically identical isolates of *H. pylori* (106). Antibiotic-resistant organisms, a resistant infection even though the organisms are sensitive *in vitro*, or both, may contribute to failures in *H. pylori* eradication therapies (107). Intracellular activity of the antibiotic is important for a successful eradication of *H. pylori* *in vitro*, indicating that intracellular organisms may be responsible for recurrent infections after treatment. Antimicrobial resistance in *H. pylori* is emerging, especially resistance to nitroimidazoles (metronidazole), and is now as high as 10–50% in certain countries in Europe and the USA (108). In developing countries, almost all of the isolated strains are resistant to metronidazole. The high resistance to metronidazole has been linked to the use of this drug in the

treatment of parasitic infections in developing countries, and with its use in the treatment of dental and vaginal infections in developed countries. Resistance to the antibiotic may also appear during treatment of *H. pylori* infection, which further contributes to the increasing antibiotic resistance.

Up to 15% of isolated strains from developed countries are resistant to the macrolide clarithromycin. Secondary resistance, which occurs after eradication therapy, may increase to up to 60%. Resistance to clarithromycin is caused by a mutation in 1 of the 2 copies of the 23S rRNA gene which can be detected by PCR followed by restriction fragment length polymorphism (RFLP) of the amplified product (109). A new assay to detect clarithromycin resistance directly from biopsies showed good results for the detection of multiple genotypes, sensitive and resistant *H. pylori*, when present in the same sample (110). The emergence of a resistance mutation in 1 of 2 strains in multiple strain infection during treatment has been observed (111). Resistance to amoxicillin is very rare, but differences in susceptibility are related to the absence of certain penicillin binding proteins in the bacteria.

Vaccine development

In trials so far, potential vaccine candidates such as whole-cell extracts or sonicates of *H. pylori* containing a mixture of immunogenic outer membrane proteins, porins and adhesins, or *H. pylori* urease, catalase, VacA and heat shock proteins together with cholera toxin (CT) or *E. coli* labile toxin (LT) as adjuvants, have been tested in animal models with variable, but promising, results (104, 112, 113). No adjuvant has yet been found suitable for use in humans, and without the adjuvant, the vaccine candidate works poorly, giving a low and insufficient immune response. For a vaccine to be effective, the antigen needs to be universal among strains of *H. pylori*, and the delivery of the antigen must give a suitable response in the host without any side-effects induced by the adjuvant. Sonicates of *H. pylori* together with Freund's incomplete adjuvant (parenteral) or *E. coli* LT (oral) were recently tested in an animal model with gnotobiotic piglets (114). After challenge with *H. pylori*, the vaccination suppressed, but did not prevent, the infection, and the vaccination did not cure the infection in piglets already colonized by *H. pylori*. Oral administration of recombinant urease and LT to rhesus monkeys protected against *H. pylori* infection and did not cause any side-effects (45). A vaccine study with orally administered urease and LT to *H. pylori*-infected human volunteers has been performed with similar results: the vaccine is immunogenic in humans and the combination of urease and LT is well tolerated (115).

The antigen that may be included in a final vaccine used in humans needs to be effective when used as a single component, should give an additive or synergistic effect together with urease, needs to be easily produced on a

large scale and easily purified in large amounts, needs to be non-toxic and should not give cross-reactions with human tissue antigens, and needs to be conserved among *H. pylori* isolates from different geographic regions. *Helicobacter pylori* LPS which contain cross-reactive Lewis antigens, and the CagA protein which is only produced by a subset of strains, are therefore not useful in a vaccine. Future vaccine candidates may be found by proteome techniques, including 2-dimensional (2D) gel electrophoresis (116, 117). The antigen may be introduced into humans by a live carrier organism (live carrier vaccination) or by intramuscular injections of *H. pylori* DNA for specific genes such as urea (DNA vaccination), which diminishes the need of mucosal adjuvants for obtaining immune responses in the host. The routes of vaccine administration are also of importance. Oral, nasal and rectal administration have been tested with positive results. For a vaccine to be successful, not only prophylactic but also therapeutic applications need to give positive test results.

DISCUSSION

Helicobacter pylori: a parasite or a commensal?

Helicobacter pylori is an 'old' microorganism which has been living together with its human host for centuries. The detection of this bacterium in patients with symptomatic gastric diseases was a breakthrough for both the treatment of peptic ulcer disease and studies of other infectious diseases (118). Infectious agents other than *H. pylori* involved in chronic inflammatory diseases and cancer have also recently been found. These findings are of major importance for public health, treatment of chronic diseases and prevention of infection, in reducing the number of infected people who are at risk of chronic disease (119). The transmission routes of *H. pylori* are still not clear, but it seems that several routes are used by the bacterium in developed and developing countries. In developed countries such as Sweden, *H. pylori* infection is rare among the young, indicating that improved childhood living conditions have halted the transmission of the bacterium within families, with a parallel decrease in symptomatic gastro-duodenal diseases, which is also partly due to an overall healthier lifestyle and a diet containing fewer preserved products. In developed countries person-to-person transmission is probably favored. In developing countries possible environmental reservoirs of the bacterium have been identified, and the transmission of *H. pylori* would certainly decrease through improvements in the quality of daily life, such as fresh water and less crowding within families. Transmission of *H. pylori* by animals to humans seems unlikely, but may occur. Extensive strain variation in *H. pylori* have been demonstrated both at the genomic and at the protein level. It has been discussed whether *H. pylori* could be divided further into subspecies, quasispecies or species complex, since the interstrain variation is higher

than in any other bacterium studied so far. By comparisons of the 2 sequenced genomes, the diversity at all levels was confirmed. Future studies will determine whether this diversity is even more evident when genomes from disease-specific strains are compared, indicating specific differences at the gene level.

Symptomatic diseases

Gastric cancer is decreasing in developed countries at the same time as *H. pylori* infection and the role of *H. pylori* in human gastric carcinogenesis are being indicated. The role of *H. pylori* in gastric MALT lymphoma is more apparent, since eradication therapy leads to the regression of the lymphoma. Infection with *H. pylori* together with other environmental factors and host factors all contribute to the development of gastric precancerous lesions, which lead to gastric adenocarcinoma. By combinations of known risk factors it is possible to predict which people with *H. pylori* infection are at high risk of developing gastric cancer. Unknown risk factors and pathogenic markers in *H. pylori* may also be of importance for the development of gastric cancer.

Differences are shown in the proportion of strains expressing the pathogenic protein markers CagA and VacA between different countries. Strains that produce these proteins seem to induce different diseases in different populations, indicating that host genetics are also of importance for disease outcome (118). In most of the studies, however, the CagA and VacA-positive phenotypes of *H. pylori* are associated with symptomatic diseases in the gastric mucosa and duodenum. Genotypic or phenotypic markers of strains that have a tendency to cause severe gastric diseases may be used to discriminate patients who should undergo eradication therapy from those who might not benefit from the disappearance of the lifelong companion *H. pylori*. Possible positive effects of the infection in persons harboring *H. pylori* are still under investigation, and several hypotheses regarding the etiology of diseases in the upper or lower part of the stomach have been proposed. *Helicobacter pylori* infection is suggested to protect persons against infection with other ingested pathogens as a result of increased gastric acidity, and to protect against gastroesophageal reflux disease (25, 120–122). It has also been found that *H. pylori* produces antibacterial peptides, to which the bacterium itself is resistant, which may kill other bacteria entering the stomach (123). To be able to separate the disease-causing infections from the silent infections is a real challenge, and one of the most important issues for therapy and prevention, in the growing research field of *H. pylori*.

An increased level of antibiotic resistance would become a severe problem if half of the world's population received treatment for the infection, and the use of the same drugs for other purposes would be arrested. In the future, vaccination may be a reality for people in both developed and

developing countries (124, 125). A vaccine that is only effective for the highly pathogenic, disease-causing, strains of *H. pylori* may become a reality if markers can be found to identify the strains that cause clinicopathological syndromes in the gastric tract. The search for genes, certain gene combinations, marker proteins and protein patterns that can be used to select patients for therapy is a hot area for research. By the 2D gel method, screening of a large number of proteins with a pool of human sera may help to identify new diagnostic and vaccine candidates (116, 117). New methods such as microarray techniques and representational difference analysis (RDA) are being used in these studies. It may be found that strains are not easily divided into types, but into a gradient of strains with increased pathogenicity.

The new millennium

The future will hopefully provide answers to many questions raised during the 1990s, since the bacterial–host interactions will be better understood as more genomes, both bacterial and human, are sequenced and ready for comparison. The transmission pathways of *H. pylori*, host vulnerability to complications from *H. pylori*, the importance of *H. pylori* infection to dyspepsia and its carcinogenic potential also need to be further studied (126–128). New vaccine candidates which specifically target the highly pathogenic strains of *H. pylori* will be identified and tested. A decrease in symptomatic *H. pylori*-related diseases and gastric cancer cases is expected as a result of decreased infection rates, new therapies for eradication of the infection, and the use of preventive and therapeutic vaccines.

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