Invited Review

Association of Helicobacter pylori with Gastroduodenal Diseases

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SUMMARY: Helicobacter pylori was first cultured in vitro in 1982. This bacterium is a spiral gram-negative rod which grows under microaerophilic conditions. The ecological niche is the mucosa of the human stomach which had been thought to be aseptic before the discovery of this bacterium. This organism causes a long-lasting infection throughout a person’s life if there is no medical intervention. Numerous persons are infected with the organism around the world, and the rate of infection in Japan is nearly 50% of the population. However, the rate of infection remains unclear because the organism has not been isolated from any environment other than several animals. H. pylori is now recognized as a causative agent of gastritis and peptic ulcers. Though gastritis, and especially chronic active gastritis, is observed at least histologically in all persons with H. pylori, peptic ulcers develop in only some infected persons. Specific factors in the host and/or the bacteria are needed for the development of peptic ulcer disease. Furthermore, H. pylori is considered to be related to the development of gastric mucosa-associated lymphoid tissue (MAIT) lymphoma, especially those of low grade. Also, H. pylori infection is a major determinant for initiating the sequence of events leading to gastric cancer. In some patients with low-grade gastric MALT lymphoma, the eradication of H. pylori led to a regression of lesion. Gastric cancer has been induced in Mongolian gerbils with long-term H. pylori infection. The combinations of drugs, which consist of an antisecretory agent (acid-suppressing agent) and antimicrobial agents, are used for the eradication of the organism. Eradication therapy is recommended at least for patients with peptic ulcers.

1. Introduction

Helicobacter pylori is a curved or spiral gram-negative bacterium, and can colonize in and under the mucus layer of human gastric mucosa without the copresence of other bacterial species. The bacterium was first cultured in Perth, Western Australia, and was first reported in 1983 as a Campylobacter-like organism (1,2). The organism was named Campylobacter pyloridis, but this was later changed to C. pylori. Subsequently, it was assigned to the new genus Helicobacter, and is now known as Helicobacter pylori (3).

The first paper relating to H. pylori described its association with gastritis (1). Researchers indicated that the organism can cause human gastritis by oral self-administration of the living organism around the world, and the rate of infection in Japan is nearly 50% of the population. However, the rate of infection remains unclear because the organism has not been isolated from any environment other than several animals. H. pylori is now recognized as a causative agent of gastritis and peptic ulcers. Though gastritis, and especially chronic active gastritis, is observed at least histologically in all persons with H. pylori, peptic ulcers develop in only some infected persons. Specific factors in the host and/or the bacteria are needed for the development of peptic ulcer disease. Furthermore, H. pylori is considered to be related to the development of gastric mucosa-associated lymphoid tissue (MAIT) lymphoma, especially those of low grade. Also, H. pylori infection is a major determinant for initiating the sequence of events leading to gastric cancer. In some patients with low-grade gastric MALT lymphoma, the eradication of H. pylori led to a regression of lesion. Gastric cancer has been induced in Mongolian gerbils with long-term H. pylori infection. The combinations of drugs, which consist of an antisecretory agent (acid-suppressing agent) and antimicrobial agents, are used for the eradication of the organism. Eradication therapy is recommended at least for patients with peptic ulcers.

2. Bacteriological features

H. pylori is a short spiral or S-shaped gram-negative rod, 0.5-0.9 μm wide and about 3 μm long, in the stomach. It is motile by means of a tuft of up to eight sheathed flagella at one pole (Fig. 1). The sheath is a kind of membrane and its good quality and uniform growth. However, H. pylori can grow in a serum-free medium containing 0.1-0.2% of dimethyl β-cyclodextrin (7,8). The growth of H. pylori in a synthetic medium was reported, but it contained large amounts of bovine serum albumin (9). In liquid culture, shaking is necessary for good quality and uniform growth.

By a time-expired culture or changes in gas conditions, H. pylori transforms to a coccoid form (Fig. 1). The coccoid form of the bacteria (coccoid body) cannot grow in any media or under any artificial conditions. However, it is reported that the oral inoculation of the coccoid body induces gastritis in mice (10). It remains unclear whether the coccoid body is in a viable but non-culturable state (VNC).

In representatives of conventional biochemical tests, H. pylori is positive for oxidase, catalase, urease, alkaline phosphatase, and γ-glutamyl transpeptidase, and is negative for nitrate reduction. The organism utilizes glucose but gives no indication of acid production. Some Helicobacter spp. have periplasmic fibers (Fig. 2), but H. pylori does not. Of these characteristics, urease is of the most importance. The urease of the organism indicates a low Km value as compared to other ureases (Table 1). Furthermore, the large content of urease...
exists at the surface of cells (17), whereas it exists only in the cytoplasm of other bacteria. Therefore, the organism exhibits very high urease activity in the whole cell, which is commonly used as a method for diagnosis of *H. pylori* infection. *H. pylori* itself is intolerant of strong acids, but the organism prefers mildly acidic conditions and can withstand a pH as low as 1.5 when urea is present (18).

*H. pylori* shows unique membrane features. On lipopolysaccharide (LPS), compared with lipid A of members of *Enterobacteriaceae*, the lipid A of *H. pylori* has an unusual composition of fatty acids (3-OH C18:0) and also a different phosphorylation pattern, with 1'- but not 4'-phosphate present in the backbone of lipid A, D-gulcosamine disaccharide (19). Because of these unique features, the endotoxic activity of the LPS is very low compared with that of *Enterobacteriaceae* (20,21). However, the LPS is recognized as an important factor in the inducement of gastritis, because the C3H/He mouse which minimally responds to LPS shows very low-grade gastritis in *H. pylori* infection compared with the C3H/H mouse which is a responder (22).

*H. pylori* has three kinds of cholesteryl glucosides (CGs) (Fig. 3) (23). CGs are very rare in animals and bacteria. In bacteria, CGs have been reported only in Mollicutes (*Acholeplasma* spp., *Mycoplasma gallinarum*, *Spiroplasma citri* and *Borrelia hermsii*). We, however, found for the first time a phosphate-linked cholesteryl glucoside like cholesteryl phosphatidyl glucoside (CPG) in *Helicobacter*. In the organism, CGs contribute about 25% by weight of the total extractable lipids. The organism cannot synthesize cholesterol in the same way as other bacteria, but appears to accumulate free cholesterol from media (24). Furthermore, CGs appear to be a unique feature of genus *Helicobacter*, because they are present in 16 of 18 *Helicobacter* species (25,26).

As mentioned above, urease is located on the outer membrane. Heat shock protein (HSP) 60 is also found in the same location. It was reported that this was synthesized in the cytoplasm and absorbed on the surface after spontaneous autolysis (27). These are major proteins in the organism.

The overall genomic organization has been found in two strains, J99 (28) and 26695 (29), of *H. pylori*. The general features are listed in Table 2. The annotated genome sequences and further information concerning strains J99 and 26695 are available on the World Wide Web site (http://www.tigr.org/tdb/mdb/hpdb/hpdb.html) and in the *H. pylori* database.
Table 1. Biochemical characters of purified ureases

<table>
<thead>
<tr>
<th>Organism</th>
<th>Km (mM)</th>
<th>Specific activity</th>
<th>Native Mr (kDa)</th>
<th>Subunit Mr</th>
<th>Subunit composition</th>
<th>Metal content</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>0.17</td>
<td>1,100</td>
<td>530</td>
<td>α = 26.6</td>
<td>(α + β)</td>
<td>1.0 Ni/β</td>
</tr>
<tr>
<td></td>
<td>-0.48</td>
<td>-1.700</td>
<td></td>
<td>β = 60.5</td>
<td>×6</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella aerogenes</em></td>
<td>2.8</td>
<td>2,200</td>
<td>224</td>
<td>α = 9</td>
<td>(α + β + γ)</td>
<td>2.1 Ni</td>
</tr>
<tr>
<td></td>
<td>-4.5</td>
<td>-180,000</td>
<td>-380</td>
<td>β = 11</td>
<td>×2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>γ = 72</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ureaplasma urealyticum</em></td>
<td>2.5</td>
<td>33,530</td>
<td>150</td>
<td>α = 66</td>
<td>α or α + β or α 6</td>
<td>2.0 Ni/α</td>
</tr>
<tr>
<td></td>
<td>-4.5</td>
<td>-180,000</td>
<td>-380</td>
<td>−76</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Jack bean (plant)</em></td>
<td>2.9</td>
<td>1,000</td>
<td>590</td>
<td>α = 98</td>
<td>α 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-5.5</td>
<td>-5,500</td>
<td></td>
<td>−76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) These data were measured with different buffers, temperatures, and pHs.
2) mol of urea/min per mg of protein
3) The small, medium and large subunits are designated as α, β, and γ, respectively.
(from refs. 11-16)

CGL (G-2): Cholesteryl-α-D-glucopyranoside (base substance)

CAG (G-1): Cholesteryl-6-O-tetradecanoyl-α-D-glucopyranoside

CPG (G-3): Cholesteryl-6-O-phosphatidyl-α-D-glucopyranoside

Fig. 3. Structures of cholesteryl glucosides of *H. pylori*.

Table 2. General features of the *H. pylori* genomes

<table>
<thead>
<tr>
<th>Feature</th>
<th>Strain 26695 (UK)</th>
<th>Strain J99 (USA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (base pairs)</td>
<td>1,667,867</td>
<td>1,643,831</td>
</tr>
<tr>
<td>G+C content (%)</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Regions of different G+C content</td>
<td>8*(5)</td>
<td>9</td>
</tr>
<tr>
<td>vacA genotype</td>
<td>sla/ml</td>
<td>slb/ml</td>
</tr>
<tr>
<td>Open reading frames (ORF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coding % of genome</td>
<td>91.0</td>
<td>90.8</td>
</tr>
<tr>
<td>Number of ORF</td>
<td>1,552*(1,590**)</td>
<td>1,495</td>
</tr>
<tr>
<td>Functionally classified</td>
<td>895</td>
<td>874</td>
</tr>
<tr>
<td>Conserved with no function</td>
<td>290</td>
<td>275</td>
</tr>
<tr>
<td><em>H. pylori</em> specific</td>
<td>367</td>
<td>346</td>
</tr>
<tr>
<td>Number with signal sequence</td>
<td>517</td>
<td>502</td>
</tr>
<tr>
<td>average length (base pairs)</td>
<td>954</td>
<td>998</td>
</tr>
</tbody>
</table>

Insertion elements (IS)

| IS605 (complete copies) | 13(5) |
| IS606 (complete copies) | 4(2)  |

RNA elements

| 23S-5S rRNA | 2 |
| 16S rRNA    | 2 |
| tRNAs       | 36 |

(referred and modified mainly from ref. 29)

* The data are recalculated in ref. 29.
** indicate the original data in ref. 28.

performed within several hours. Therefore, the combination of several methods, especially culture, a rapid urease test, and the observation of stained tissue, is usually used.

*H. pylori* infection is detected by the elevation of titers of specific IgG antibody in serum. The determination of specific IgA levels in the saliva may also be used for the diagnosis. However, the use of specific IgM levels is not helpful for diagnosis because long-term infection is usually diagnosed long after IgM levels peak, which most often occurs in youth while the infection is asymptomatic.

The urea breath test (UBT) and the detection of *H. pylori* antigens in feces are non-invasive methods. Especially, the UBT has very high sensitivity. However, 13C-urea and costly equipment are needed for the test.

4. Epidemiology

The seroepidemiology of *H. pylori* has been studied in many countries (Fig. 4). The high frequency of seropositivity...
Table 3. Methods for diagnosis of H. pylori (HP) infection

<table>
<thead>
<tr>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Culture</td>
</tr>
<tr>
<td>Grind a gastric specimen, inoculate onto agar media, and culture the media for a week under microaerophilic conditions.</td>
</tr>
<tr>
<td>Brain heart infusion agar medium with 5.7% horse blood and antimicrobials (vancomycin, trimethoprim, polymyxin B, and amphotericin B) are commonly used for the first isolation.</td>
</tr>
<tr>
<td>2. Rapid urease test</td>
</tr>
<tr>
<td>(Commercial kits indicating positive within several hours are available.)</td>
</tr>
<tr>
<td>Inoculate gastric specimen (grinded or not) to kits containing urea and a pH-indicator. The color will change with ammonia production by strong urease activity when HP exists in the tissue.</td>
</tr>
<tr>
<td>3. Histological examination</td>
</tr>
<tr>
<td>Stain formalin-fixed tissues by several methods.</td>
</tr>
<tr>
<td>Detection of HP in the tissue stained with hematoxylin-eosin is quite hard. Giemsa stain, Warthin-Starry stain, Genta stain, stain with acridine orange, and immunostaining are used.</td>
</tr>
<tr>
<td>4. Dye-spraying endoscopy</td>
</tr>
<tr>
<td>Perform endoscopy in patients administered with acid-suppressing agents.</td>
</tr>
<tr>
<td>Spray liquid containing urea and dye (phenol red or a dye from red cabbage) to the gastric mucosa. The color of the HP-positive area will change by the same reaction as 2).</td>
</tr>
<tr>
<td>5. PCR (Polymerase chain reaction)</td>
</tr>
<tr>
<td>Detect HP-specific genes using PCR in the gastric tissues.</td>
</tr>
<tr>
<td>Primers to genes of the urease or 16S rRNA are usually used.</td>
</tr>
<tr>
<td>The PCR can be applied to saliva or feces.</td>
</tr>
<tr>
<td>6. Detection of anti HP IgG antibody</td>
</tr>
<tr>
<td>(Commercial kits are available.)</td>
</tr>
<tr>
<td>Various antigens (sonicated bacteria, acid-extracts, urease, etc.) are used.</td>
</tr>
<tr>
<td>7. Urea breath test (UBT)</td>
</tr>
<tr>
<td>Give liquid containing 13C-urea to patients orally.</td>
</tr>
<tr>
<td>13C-carbon dioxide is produced from urea by the same reaction as 2).</td>
</tr>
<tr>
<td>The carbon dioxide is absorbed in the intestine and is exhausted in the lung. Measure content of 13C-carbon in breathing 15-30 min after the administration.</td>
</tr>
<tr>
<td>8. Detection of HP-antigens in feces</td>
</tr>
<tr>
<td>(A commercial kit is available)</td>
</tr>
<tr>
<td>HP-antigens are detected with polyclonal antibody in ELISA.</td>
</tr>
</tbody>
</table>

5. The association between H. pylori and gastroduodenal diseases

Many gastroduodenal diseases are now recognized to be associated with H. pylori infection. The disease associations are summarized below.

A. Gastritis

A-1) acute gastritis (first infection)

In most cases of H. pylori infection, the causal events are not well known because the infection has been long-standing by the time of diagnosis. Most cases of childhood infection may be asymptomatic. However, H. pylori infection was determined by seroconversion or culture-positive in children who exhibited abdominal pain (36,37). Acute gastritis was caused in volunteers (researchers) who ingested the living organism. When some people who exhibited a normal gastric mucosa and were free of the organism at the time of their first endoscopy developed abdominal pain 1-2 weeks after the endoscopy, acute gastritis (acute gastric mucosal lesion; AGML) was found and the organism was detected in the second endoscopy (38,39). Based on these data, H. pylori can cause acute gastritis.

One feature of the onset of infection is the disappearance of acid secretion for a few months (40,41). Clinical signs of acute gastritis, especially AGML, disappear 1-2 weeks after onset. However, in most people infected with H. pylori, the infection is life-long, and acute gastritis proceeds to chronic gastritis. However, it was reported that the organism disappeared spontaneously 3-4 weeks after the onset of AGML in some people infected by endoscopy or after the onset of acute gastritis in a volunteer’s infection (42,43).

A-2) chronic gastritis

Most people with H. pylori infection do not exhibit any clinical signs. However, the presence of the organism is always histologically associated with chronic active gastritis, and its eradication is followed by the resolution of gastritis. Acute gastritis proceeded to chronic gastritis with clinical symptoms in the volunteers mentioned above. In addition, the oral administration of the living organism causes chronic gastritis in animals (mice, mongolian gerbils, piglets, monkeys). Among these animals, the mongolian gerbil has the highest sensitivity to colonization by the organism and shows the most severe gastritis.
In chronic gastritis with *H. pylori*, infiltrations of chronic inflammatory cells (lymphocytes and plasma cells) and neutrophil polymorphs are present in the gastric mucosa. The term of chronic active gastritis indicates the period in which an infiltration of neutrophil polymorphs has occurred. Damage to the surface epithelium is a feature of chronic active gastritis. Lymphoid follicles (aggregates of B-lymphocytes with germinal centers) are often observed in the mucosa of infected persons, especially children, whereas uninfected gastric mucosa contain minimal lymphocytes. These lymphoid follicles are a kind of acquired MALT (mucosa-associated lymphoid tissue) - as opposed to Peyer’s patches which are normally present. The levels of chronic inflammatory infiltrates correlates closely with the density of the bacterial colonization. Generally, the infiltration originates in the antrum and proceeds to the corpus.

**A-3) atrophic gastritis and other changes (intestinal metaplasia and gastric metaplasia of the duodenum)**

Atrophy in the mucosa of the stomach is caused by a loss of glandular tissue. Repeated tissue injury causes atrophy and leads to a thinning of the gastric mucosa, which induces hypochlorhydria. In patients with chronic gastritis induced by *H. pylori* infection, the prevalence and severity of glandular atrophy increases steadily with age. Patients without *H. pylori* infection rarely develop atrophic gastritis. *H. pylori* inhabits the gastric mucosa, including the mucus layer. The loss of glandular tissue and the thinning of the mucosa reduce the density of the organism. In Japan there are many patients with severe atrophic gastritis.

Intestinal metaplasia is frequently observed in chronic *H. pylori* gastritis. The report (44) suggests that *H. pylori* infection and bile reflux act synergistically to produce intestinal metaplasia. The intestinal epithelium (intestinal metaplasia) is resistant to bile acids, and is not colonized by the organism. The definition of gastric metaplasia of the duodenum is the appearance of gastric mucin cells on the surface of the duodenal mucosa. Gastric metaplasia is also observed in duodenal Crohn’s disease. This condition can be induced in rats by repeated injections of gastrin (45), and is not seen in patients with achlorhydria (46). Therefore, gastric metaplasia is probably a non-specific response to injury by acid. *H. pylori* can colonize gastric mucin cells and can be detected only at the sites of gastric metaplasia in the duodenum.

**A-4) pathogenic mechanisms in gastritis**

*H. pylori* reaches the gastric mucosa, the ecological niche of the organism, through the gastric juices, adheres to the mucosa, and colonizes it. The sheathed flagella and the urease are considered to be critical factors for colonization because mutant strains did not colonize the mucosa in the stomach of the mouse (47–49). The sheathed flagella would indicate motility without a degradation in the acid environment of the gastric juices, and the urease which show the lowest Km values would neutralize the acidic microenvironment around the organism, utilizing a low concentration of urea in the gastric juices.

Adhesion is an essential step for infection by all pathogens. For *H. pylori*, many adhesins and their receptors have been reported (Table 4). The organism adheres well not only to human gastric cell lines, but also to other cell lines. Among these adhesins and receptors, it is not known which are actually important for adhesion to occur in the human gastric mucosa.

The changes in acute gastritis (first infection) are likely due to direct actions of bacterial products. However, in addition to the direct action, immune responses appear to play an important role in chronic gastritis.

Many virulence factors were nominated, but it is not known which are important. The organism has cholesterol glucosides, LPS, urease and HSP 60, and produces phospholipase and mucinase. The cholesterol glucosides indicate hemolytic activity (23). Ammonia, which is produced from urea by the urease, can cause mucosal damage (67,68). Furthermore, monochloramine, which is produced by the reaction between ammonia and chloride ions in the presence of oxygen free radicals formed by neutrophils, is also toxic to cells (69).

*H. pylori* also produces and releases a vacuolating cytotoxin (VacA) which produces vacuoles in many cell lines (Fig.5) (70). The vacA gene encodes a precursor protein of 139.6 kDa consisting of a 33-amino acid signal sequence, the 87-kDa cytotoxin and a 50-kDa C-terminal domain (71). The secretion of the cytotoxin through bacterial membranes is considered as follows: the precursor protein passes through the bacterial cytoplasmic membrane by a sec-dependent pathway; the 50-kDa C-terminal domain is inserted into the outer membrane; and the cytotoxin domain is cleaved off and released into the supernatant (71). Intra-gastric administration of VacA to mice caused some tissue damage resembling that found in patients with *H. pylori* infection (72). Furthermore, VacA may not be the only virulence factor, because three proteins which show moderate similarities (26-31%) to the carboxy-terminal end of VacA exist in strain 26695 (28).

*Most* *H. pylori* are free-swimming in the mucosa, but some

<table>
<thead>
<tr>
<th>Adhesins</th>
<th>Receptors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 kDa protein (HpA)</td>
<td>N-acetylgalactosaminyl lactose</td>
<td>50-52</td>
</tr>
<tr>
<td>63 kDa protein</td>
<td>phosphatidyethanolamine</td>
<td>53-55</td>
</tr>
<tr>
<td>(exoenzyme S-like adhesin)</td>
<td>gangliotriaosylceramide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>gangliotetraosylceramide</td>
<td></td>
</tr>
<tr>
<td>19.6 kDa protein</td>
<td>laminin</td>
<td>56</td>
</tr>
<tr>
<td>25 kDa protein</td>
<td>laminin</td>
<td>57</td>
</tr>
<tr>
<td>75 kDa protein (Bab A)</td>
<td>Lewis B antigen</td>
<td>58,59</td>
</tr>
<tr>
<td>61 kDa protein</td>
<td>H type 2, Lewis A, Lewis B antigens</td>
<td>60</td>
</tr>
<tr>
<td>16 kDa protein</td>
<td>Lewis X antigen, mucin</td>
<td>61</td>
</tr>
<tr>
<td>59 kDa hemoagglutinin</td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>60 kDa heat shock protein (HSP B)</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>GM3, lactosylceramide sulfate</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>GM3, sulfatides</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>heparan sulphate</td>
<td>66</td>
</tr>
</tbody>
</table>
of the organisms adhere to gastric epithelial cells. This adhesion induces cytoskeletal rearrangements in AGS cells (a human gastric adenocarcinoma cell line) and tyrosine phosphorylation of the cell proteins, resembling that of enteropathogenic E. coli (73). Further, the adhesion of the bacteria to gastric epithelial cell lines induces IL-8 expression and secretion in the cells. IL-8 attracts and activates neutrophils and exhibits various bioactivities as a proinflammatory chemokine. The cag pathogenicity island (cag PAI) is a gene cluster, and the products of cag PAI are considered to be major proteins which induce IL-8 expression in the cells (74). The G+C percentage of cag PAI is low (35% in strain 26695) compared to that of the whole genome (39% in strain 26695), and this gene cluster is likely acquired horizontally (28,29). CagA (the product of cytotoxin-associated gene A) is a 120-128 kDa protein. Although CagA was formerly thought to be associated with VacA, these two proteins are found to be independent. Furthermore, CagA does not affect the induction of IL-8 expression in the epithelial cells, although previously it was thought to induce IL-8 expression. The products of genes other than cagA (cagE, G, H, I, L, M) in cag PAI are found to affect the induction of IL-8 expression (74).

Strains which do not exhibit VacA activity (the vacuolation of epithelial cells by the addition of a culture-supernatant to a cell-line culture) or are missing the cagA gene are also isolated from patients with chronic H. pylori gastritis. Thus, VacA and CagA (cag PAI) are not critical factors for the induction of gastritis. These might play more important roles in peptic ulcer diseases.

Much research concerning immune responses including cytokine production, has been carried out. Only some recent works are summarized here. In the gastric mucosa of mice with Helicobacter infection (H. pylori or H. felis), the TH1 (T helper cell 1) response is dominant in CD4-T cells (75). It was reported that TH1 cells enhanced gastritis, and that TH2 cells reduced the bacterial load (76). In human gastric mucosa of patients with chronic H. pylori gastritis, the TH1 phenotype is also dominant (77,78). Certainly, the TH1 response is likely to enhance gastritis. Among TH1 responses, gamma interferon (INF-γ) appears to play an important role in gastric inflammation because H. pylori which colonizes the IFN-γ gene knockout mice, does not cause gastric inflammation (79).

B. peptic ulcer diseases [duodenal ulcer (DU) and gastric ulcer (GU)]

H. pylori infection rates in patients with peptic ulcers are high. A report in Japan mentioned that the infection rate was 97% in patients with DUs and 98% in patients with GUs, whereas it was about 40% in asymptomatic persons (80). In an animal infection model (mongolian gerbil), the development of GU was observed (81). The eradication of the organism in patients with peptic ulcers suppresses the recurrence of ulcers (82,83). The recurrence rate a year after therapy is below 20% in the eradicated patients, and over 50% in those without eradication. These data, especially the latter, clearly indicate that H. pylori is the major cause of peptic ulcers. The NIH Consensus Development Panel on H. pylori in Peptic Ulcer Disease in the United States recommended the eradication of the pathogen in patients with peptic ulcers (84). However, peptic ulcer diseases are caused only in part by H. pylori infection. Other drugs, especially non-steroidal anti-inflammatory drugs (NSAIDs), also cause peptic ulcer diseases. Therefore, additional or specific factors in the host and/or the bacteria are certainly needed for the development of peptic ulcer diseases.

The mechanisms concerning the development of peptic ulcers in patients with H. pylori infection are not clear. Acid secretion plays an important role both in GU and DU diseases, although the pathosis in each ulcer disease is different. In patients with DU, acid secretion persists for a longer period after meals (85,86) and gastrin release is stimulated (87,88), whereas somatostatin decreases (89,90). Patients with GU secrete, on average, less acid than controls (91). However, acid secretion also plays an important role in GU disease because ulcer healing is faster and recurrence is less when acid secretion is suppressed (92). Detailed mechanisms and associations based on clinical findings are not included in this review.

H. pylori is sensitive to bile salts (93), although the organism was detected at sites of gastric metaplasia in the duodenum. Unconjugated bile salts are more toxic to H. pylori than the conjugates (93). Bile salts without taurine conjugates are precipitated in an acidic environment. Taurine conjugates have only low toxicity to the organism. One report (94) mentioned that the precipitation of bile salts without taurine conjugates in acid would allow the colonization of the duodenal bulb. The colonization at the sites of gastric metaplasia in the duodenum may be key to the development of duodenal ulcers.

In terms of additional or specific factors in the host, cigarette smoking, blood group O and non-secretors, inherited factors, etc. have been reported. In inherited factors, it was reported that HLA-DQA1 was associated with peptic ulcer diseases (Persons having DQA1*0102 are resistant and those having DQA1*0301 are sensitive.) (95). VacA and cagPAI (cagA) are considered to be the representative specific factors in the bacteria. The vacA gene is classified by the sequence at the sites of the signal sequence and the middle region to s1 and s2, and m1 and m2, respectively (96). Strains of type s1/m1 show the high biological activity of VacA (96). Strains in Europe and the U.S.A. that patients with ulcers were infected with strains of type s1/m1 more often than with other types, compared with non-ulcer patients (96,97). However, this difference in the type distributions of the vacA gene between the strains from ulcer and non-ulcer patients was not observed in Japan, because the type of vacA gene is s1/m1 in most strains (98,99). Conversely, it was also reported in Europe and the U.S.A. that patients with ulcers were infected with strains having the cagA gene more often than with other types, compared with non-ulcer patients (100). However, in
Japan the differences in the rates of possessing cagA-positive strains between ulcer and non-ulcer patients with *H. pylori* infection were of little import because most strains in this region have the cagA gene (101).

### C. Gastric MALT lymphoma

Isaacsen developed the concepts of gastric MALT and MALT lymphoma (MALToma) (102,103). A MALToma is generated from MALTs, which are collections of B-cells. The MALToma is classified as a Marginal zone B-cell lymphoma in peripheral B-cell neoplasms in the Revised European-American Classification of Lymphoid Neoplasms of 1994 (104). A disease formerly referred to as reactive lymphoreticular hyperplasia is considered to be included in the category of MALTomas. It remains debatable whether all MALTomas are strictly neoplasmas.

Most patients with gastric MALToma are infected with *H. pylori* (92% in ref. 105). MALTs are often observed in the gastric mucosa with *H. pylori* infection. Furthermore, some low-grade MALTomas disappear or regress after the eradication of *H. pylori* (106,107). Therefore, *H. pylori* infection appears to be associated with gastric MALToma. However, some low-grade MALTomas and most high-grade MALTomas do not exhibit any regression after eradication of the organism (108,109).

Although the association of *H. pylori* with lymphomagenesis of gastric MALToma is unclear, the pathosis of this disease, especially in relationship to low-grade cases, appears to be associated with the immune response against the HSP 60 family. Follicular dendritic cells of germinal centers in the gastric mucosa affected by low-grade MALToma were immunostained positively with anti-*H. pylori* polyclonal antibodies and with anti-human HSP 60 monoclonal antibody (110). The antibody titer to the recombinant human HSP 60 were observed to be significantly elevated only in patients with gastric MALToma among gastric diseases with *H. pylori* infection (111).

Conversely, human lymphocytes can be transplanted to severe combined immunodeficient (SCID) mice that have neither mature T- nor B-cells. One month after peripheral blood mononuclear cells from *H. pylori* infected patients with gastric MALTomas were transplanted to SCID mice, oral administration of live *H. pylori* to the SCID mice led to gastric ulcers within 3 days, most commonly by day 1 (112) (Fig.6). Gastric ulcers and erosions are occasionally observed in patients with the disease. These data suggest that host immune responses against *H. pylori* are involved in the development of gastric ulcers in patients with gastric MALTomas.

### D. Gastric cancer

Even though the number of patients with gastric cancer in Japan is decreasing, there are still many patients with gastric cancer in Japan. Early gastric cancers develop in areas of intestinal metaplasia or chronic atrophic gastritis, which are observed frequently in patients with long-term *H. pylori* infection. Many groups have indicated significantly higher seroprevalences of *H. pylori* in patients with gastric cancer in case-control studies (113-115), although in some studies there are no differences between the seroprevalence in positive cases and controls (116,117). Strong evidence for an association between *H. pylori* infection and the development of gastric cancer has come from prospective (cohort) studies (Table 5).

In 1994, the World Health Organization added *H. pylori* to its list of known carcinogens (121). Furthermore, gastric cancer has been induced in Mongolian gerbils with long-term *H. pylori* infection (122-124). Now *H. pylori* infection is regarded as a major determinant in the first stage of the sequence of events leading to gastric cancer. It has been estimated that nearly 80% of gastric cancers may be attributable to *H. pylori* infection.

*H. pylori* is epidemiologically associated with the intestinal type (well-differentiated type) but not the diffuse type of gastric cancer at the antrum and the corpus (125), and is not associated with cancers of the gastric cardia. In contrast, Epstein-Barr virus is considered to be associated with most lymphoepithelial-like gastric carcinoma and nearly 10% of typical type cancers (differentiated and diffuse) involving the corpus and the cardia of the stomach (126).

There is no evidence that *H. pylori* infection is, in itself, directly genotoxic or mutagenic. A number of ideas regarding carcinogenic mechanisms have been proposed. First, a significant decrease in ascorbic acid in the gastric juices is observed in patients with chronic *H. pylori* gastritis (127,128), although the reason is unclear. Ascorbic acid is an antioxidant which indicates functions as a scavenger of reactive oxygen and an inhibitor of N-nitrosation. A reduction of these chemopreventive effects may allow the development of cancer. Second, *H. pylori* infection increases the rates of gastric epithelial cell turnover, which may result as an effect of the ammonia produced by urease. Cell division is vital to the

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**Table 5. Prospective studies of the association between *H. pylori* (HP) and the development of gastric cancer**

<table>
<thead>
<tr>
<th>Region</th>
<th>Follow-up (Mean/year)</th>
<th>cancer HP positive</th>
<th>non-cancer HP positive</th>
<th>odds ratio</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>British &amp; Welsh</td>
<td>6</td>
<td>20/29 (69%)</td>
<td>54/116 (47%)</td>
<td>2.8</td>
<td>118</td>
</tr>
<tr>
<td>American (California)</td>
<td>14</td>
<td>92/109 (84%)</td>
<td>66/109 (61%)</td>
<td>3.6</td>
<td>119</td>
</tr>
<tr>
<td>Japanese-American (Hawaii)</td>
<td>13</td>
<td>103/109 (94%)</td>
<td>83/109 (76%)</td>
<td>6.0</td>
<td>120</td>
</tr>
</tbody>
</table>

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development of cancer.

6. Treatment

_**H. pylori**_ is sensitive to many antimicrobial agents in vitro, though it is resistant to trimethoprim, polymyxin B, and vancomycin which are contained in the medium used for the first isolation. However, it is difficult to eradicate the organism from the stomach with a single use of an effective antimicrobial agent. It is generally accepted that a combination of at least two, and possibly three, effective drugs should be given for a minimum of 1 week to eradicate the organism. All combinations contain an antisecretory agent and antimicrobial agents.

The administration of antisecretory agents increases the pH in the stomach. At a low pH, the MICs (minimum inhibitory concentration) of most antimicrobial agents increase significantly (129) compared with at neutral pH. The administration of antisecretory agents secures the efficacy of antimicrobial agents in addition to an acceleration of the cure of ulcers and erosions. Among antisecretory agents, proton pump inhibitors (PPIs) (H^+-K^+ ATPase antagonist) are usually used in eradication therapies, and these inhibitors have a stronger antisecretory effect than histamine H2 receptor antagonists. Furthermore, PPIs show direct antimicrobial effects against _**H. pylori**_ in vitro, but not against other bacteria (130) (Histamine H2 receptor antagonists do not show this effect). Although PPIs inhibit urease (131), this activity is found to have no relation to the anti- _**H. pylori**_ effect (132), and the mechanism of the effect is unclear. It is controversial whether the anti- _**H. pylori**_ effect in vitro is practically effective in vivo in eradication therapies.

Among antimicrobial agents, representatives in the latest eradication therapies are amoxicillin (AMPC), clarithromycin (CAM), and nitroimidazoles [metronidazole (MTZ) or tinidazole]. _**H. pylori**_ rapidly acquires resistance to some antimicrobial agents. Acquired resistance by mutations to CAM have been demonstrated (133,134). Plasmid-mediated resistance has not been reported in any drug. Nitroimidazoles are widely used to treat anaerobic bacteria and protozoa such as _Trichomonas_ and _Giardia_. The higher frequencies and doses produce higher rates of resistance. The rate of resistance in isolated strains of _**H. pylori**_ is about 80% in Africa and 30% in the U.S.A. CAM, a new macrolide, is administered to patients with other infectious diseases, especially respiratory infectious diseases. The rate of resistance is over 10% in Japan (135). Few resistant strains to AMPC have been found, although AMPC has been used for a long period throughout the world. However, a stable strain of _**H. pylori**_ resistant to AMPC was reported in the Netherlands in 1998 (136).

7. Disease associations of _**H. pylori**_ infection with extra-gastrodoudenal diseases

The curing of _**H. pylori**_ infection may provoke reflux esophagitis (137,138). Acid secretion is low in patients with severe corpus gastritis. Successful eradication of _**H. pylori**_ increases acid secretion, which may cause reflux esophagitis (gastroesophageal reflux diseases).

_**H. pylori**_ infection may be associated with hyperammonaemia. Hyperammonia in patients with severe liver damage is considered to be produced by bacterial urease in the intestine. _**H. pylori**_ also produces ammonia by means of its strong urease activity. The rate of hepatic encephalopathy in acute alcoholic hepatitis is reported to be higher in patients with _**H. pylori**_ infection (139). In some patients with cirrhosis, ammonia levels are significantly decreased after the eradication of _**H. pylori**_ (140).

Recent studies have suggested that chronic infections with _Chlamydia pneumoniae_ and cytomegalovirus are associated with an increased risk of coronary artery disease and atherosclerosis. Some reports suggested that _**H. pylori**_ infection is also associated with these diseases (141,142), but this remains controversial (143).

8. Associations of helicobacters without _**H. pylori**_ with human diseases

Many species or strains/groups are listed in genus _Helicobacter_ (Table 6). _**H. helimannii**_ (Gastrospirillum hominis) is a spiral bacteria larger than _**H. pylori**_ and is urease-positive. It has been detected in the human stomach and is thought to cause about 1% of human gastritis. This bacteria has not been cultured in vitro, but it can grow readily in the mouse stomach. Analysis has revealed a difference in the 16S ribosomal RNA gene, resulting in its classification as a new species, _Helicobacter helimannii_. In patients with _**H. helimannii**, the rapid urease test indicates positive, but the culture using media for _**H. pylori**_ isolation is negative.

*H. fenneliae*, *H. cinaedi*, _**H. pullorum**, _**H. canis**, and _**H. rappini**_ (Flexispira rappini) have been isolated from the stools of patients with diarrhea. Most of these patients are immunodeficient. _**H. westmeadii**_ and _Helicobacter_ species strain Mainz have been isolated from the blood of patients with immunodeficiency. Conversely, some species such as _**H. hepaticus**, _**H. canis**, _**H. cholecystus**_, _**H. pullorum**, and _**H. bilis**_ have been isolated from animal livers and/or bile. Especially, _**H. hepaticus**_ has been isolated from inbred strains of mice with hepatitis, and is considered to increase the risk of hepatic carcinoma. PCR amplicons of _Helicobacter_-specific 16S ribosomal RNA primers (_**H. bilis**, _**H. pullorum**, and _**H. rappini**_) have been detected in samples obtained from human patients with chronic cholecystitis (174). These _Helicobacter_ spp. mentioned in this paragraph may frequently enter the human intestine, or may be members of a group constituting a small part of the normal intestinal flora in humans.

**REFERENCES**

Table 6. A list of species in genus Helicobacter

<table>
<thead>
<tr>
<th>Species</th>
<th>Hosts</th>
<th>Sites</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>H. pylori</em></td>
<td>human</td>
<td>stomach</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>(monkey, cat, dog)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. <em>H. cinaedi</em></td>
<td>human, hamster</td>
<td>intestine,</td>
<td>144,145</td>
</tr>
<tr>
<td><em>(H. sp. CLO-1)</em></td>
<td></td>
<td>blood (human)</td>
<td></td>
</tr>
<tr>
<td>3. <em>H. felis</em></td>
<td>human (AIDS)</td>
<td>intestine</td>
<td>144,145</td>
</tr>
<tr>
<td><em>(H. sp. CLO-2)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. <em>H. westmeadi</em></td>
<td>human (AIDS)</td>
<td>blood</td>
<td>146</td>
</tr>
<tr>
<td>5. <em>H. nemestrinae</em></td>
<td>monkey, pig tailed</td>
<td>stomach</td>
<td>147</td>
</tr>
<tr>
<td>7. <em>H. bizzozernonis</em></td>
<td>dog</td>
<td>intestine</td>
<td>149</td>
</tr>
<tr>
<td>8. <em>H. salmonis</em></td>
<td>dog</td>
<td>intestine</td>
<td>150</td>
</tr>
<tr>
<td>9. <em>H. felis</em></td>
<td>cat, (dog)</td>
<td>stomach</td>
<td>151,152</td>
</tr>
<tr>
<td>10. <em>H. acuminata</em></td>
<td>cheetah</td>
<td>stomach</td>
<td>153,154</td>
</tr>
<tr>
<td>11. <em>H. mustelae</em></td>
<td>ferret</td>
<td>stomach</td>
<td>155,156</td>
</tr>
<tr>
<td>12. <em>H. cholecystis</em></td>
<td>Syrian hamster</td>
<td>gallbladder</td>
<td>157</td>
</tr>
<tr>
<td>13. <em>H. muridurum</em></td>
<td>mouse, rat</td>
<td>intestine, (stomach)</td>
<td>158,159</td>
</tr>
<tr>
<td>14. <em>H. trogontum</em></td>
<td>rat</td>
<td>intestine</td>
<td>160</td>
</tr>
<tr>
<td>15. <em>H. rodentium</em></td>
<td>mouse</td>
<td>intestine</td>
<td>161</td>
</tr>
<tr>
<td>16. <em>H. hepatitis</em></td>
<td>mouse</td>
<td>intestine, liver</td>
<td>162</td>
</tr>
<tr>
<td>17. <em>H. bilis</em></td>
<td>mouse</td>
<td>intestine, liver,</td>
<td>163</td>
</tr>
<tr>
<td>18. <em>H. suncus</em></td>
<td>house musk shrew</td>
<td>stomach</td>
<td>164</td>
</tr>
<tr>
<td>19. <em>H. paminostensis</em></td>
<td>bird, gall, turn</td>
<td>intestine</td>
<td>165</td>
</tr>
<tr>
<td><em>(H. sp. Bird-A)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. <em>H. pullorum</em></td>
<td>bird, chicken, (human)</td>
<td>intestine, liver</td>
<td>166</td>
</tr>
<tr>
<td><em>(Flexispira raptini)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(Gastrospirillum hominis)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. <em>H. helminthii</em></td>
<td>human</td>
<td>stomach</td>
<td>169-171</td>
</tr>
<tr>
<td><em>(Gastrospirillum hominis)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. <em>H. sp. Mainz</em></td>
<td>human (AIDS)</td>
<td>blood, joint</td>
<td>172,173</td>
</tr>
<tr>
<td><em>(Campylobacter) pylori</em></td>
<td></td>
<td>intestine</td>
<td>144,145</td>
</tr>
<tr>
<td>24. <em>H. sp. CLO-3</em></td>
<td>human (homosexual)</td>
<td></td>
<td></td>
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<tr>
<td>25. <em>H. sp. Bird-B</em></td>
<td>bird, turn</td>
<td>intestine</td>
<td>165</td>
</tr>
</tbody>
</table>

*: The former name

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