

# COMMEMORATIVE LECTURE

## *Helicobacter pylori*: past, present and future

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### Introduction

Thank you very much, Professor Ishii. It is a great pleasure to be at Keio University again in such nice fine weather. I know I will enjoy my visit and your hospitality as I have done in the past.

In my lecture today, I will firstly cover the initial observations of *H. pylori* because controversies that developed in 1983 are still relevant to *H. pylori* today.

To the young investigators in the audience, I suggest that you should always keep your rejection letters. One from my collection reads, "Dear Dr. Marshall, I regret that your research paper was not accepted for presentation. The number of abstracts we receive continues to increase. For this meeting 67 were submitted and we could only accept 56." So you can imagine that in 1983 we were very depressed about our first failed attempt to present our work on *H. pylori*.

For the next 10 years, most gastroenterologists believed that *H. pylori* was a commensal. To demonstrate that *H. pylori* was a pathogen, we had to show 1) an association with disease, 2) evidence for an immune reaction, 3) an improved clinical outcome after eradication 4) an absence of benefit and 5), that healthy people did not have *H. pylori*.

The fifth task was a problem because epidemiologic studies revealed that many people – 50 percent of the Japanese population for example – carried *H. pylori*, but were apparently healthy.<sup>1</sup> Although the pathologist looking at the biopsy of the stomach can see that gastritis is present, it is true that most people with *H. pylori* are clinically well. This fact is still the main paradox for *H. pylori* disease.

Dr. Robin Warren's initial observation of curved gastric bacteria was made on 12th June 1979 (his 40th birthday). A photocopy of his original histology report from that day is published in the book called *Helicobacter*

*Pioneers: Firsthand Accounts from the Scientists Who Discovered Helicobacters 1892–1982*.<sup>2</sup>

Initially the new bacterium was the only one of its type, so we allocated it to a genus that already existed, the *Campylobacters*. Originally the new bacterium was called *Campylobacter pyloridis*, but since then many similar organisms have been found in animals, so the new genus of *Helicobacter* was created to reflect the spiral or helical forms.<sup>3</sup> Typical examples are *H. felis* (from cats), *H. mustelae* (from ferrets), and *H. bizzeronii* (from dogs). *Helicobacter bilis* is a pathogen of laboratory mice and causes hepatitis.<sup>4</sup>

There are occasional reports linking other *Helicobacters* to human disease. For example, *H. pullorum* and *H. bilis* associated with gall-bladder cancer in one Chilean PCR study.<sup>5</sup> *H. cinaedi*, a commensal of hamsters, has been shown to cause rare cases of colitis in immunosuppressed patients.<sup>6</sup> However, these reports are often difficult to substantiate, and, although new *Helicobacters* are being described annually, *H. pylori* is still the most important proven pathogen of the genus. Today it is quite straightforward to classify new *Helicobacters* by their 16S RNA sequence.

### Diagnosis

Initial diagnosis of *H. pylori* is now most commonly made at endoscopy with the use of rapid urease test, histology, and culture. Non-invasive tests include serology for IgG, breath tests, faecal antigen tests, and the urine antibody test.

Other than presence of ulcers, the endoscopic appearance most predictive of *H. pylori* is the "goose-flesh" or "chicken skin" appearance of the antral mucosa. This had been reported in the pediatric literature even before *H. pylori* was discovered.<sup>7</sup> In many

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patients with *H. pylori* though, the stomach appears 100% normal.

In most laboratories, the toluidine blue or Giemsa stain can be used to demonstrate *H. pylori* in gastric tissue. The gastric histology related to *H. pylori* is called active chronic gastritis. If the gastroenterologist takes the biopsy from the border of the ulcer, inflammation caused by the healing process might be confused with gastritis. So the diagnostic biopsy for *H. pylori* should be taken three or four centimeters away from the ulcer. Gastritis is present in 100 percent of patients with *H. pylori*, regardless of toxin production. All *H. pylori* infections cause gastritis but quite often the infected stomach appears normal endoscopically.

For the clinical microbiologist, *H. pylori* is easily identified as a gram negative curved organism that is catalase, oxidase and rapid urease positive. As with *Staphylococcus aureus*, the presence of strong catalase activity may protect *H. pylori* because catalase-positive organisms survive longer after phagocytosis.

In Australia, we diagnose 200,000 cases of *H. pylori* per annum using the same methods which are used in Japan. We believe there are four million infected persons in Australia – about 20% of the population.<sup>8</sup> Comparison of various diagnostic tests reveals that serology is the simplest test for general practice. In Australia serology costs about AUD\$25. The urea breath test (UBT) is simple but, of course, the patient has to be fasting, so it is not always possible to do the test immediately. In most countries the urea UBT is reimbursed for patients with a history of peptic ulcer and costs AUD\$65. Feces antigen is a convenient test for small children, but adult patients prefer the breath test, for about the same cost. For the gastroenterologist, the biopsy urease test costs about AUD\$10 but it gives results in one hour. The rapid result makes the test very useful as treatment may be commenced immediately. Culture takes seven days and costs about AUD\$80. Histology takes about two days and costs about AUD\$100. The advantage of histology, especially in research, is that it does provide a permanent record. You can cut further sections, perform immunological stains, or even extract DNA for molecular studies. The combination of histology and a rapid urease test, such as a CLOtest<sup>®</sup>, is the most widely accepted reference method for any *H. pylori* diagnostic study. Specimens preserved in CLOtests<sup>®</sup> can also be used for later PCR studies. So if the doctor only performs a urease test, you can use that specimen for PCR, even after many months.

### Epidemiology

In Australia and most developed countries, *H. pylori* infection before the age of 15 years is uncommon. The

increased prevalence of *H. pylori* in older persons, as high as 60% in Japan, is caused by infection acquired in childhood by the faecal oral route, before 1960 when hygiene was less perfect than it is today.<sup>10</sup>

### Pathophysiology

Addressing mechanisms of the pathogenesis, I was often asked, “How does *H. pylori* cause a duodenal ulcer? After all, most of the *H. pylori* is in the stomach.”

Several anatomists had reported that the gastric mucosa could grow into the duodenum where it was referred to as “gastric metaplasia”.<sup>9</sup>

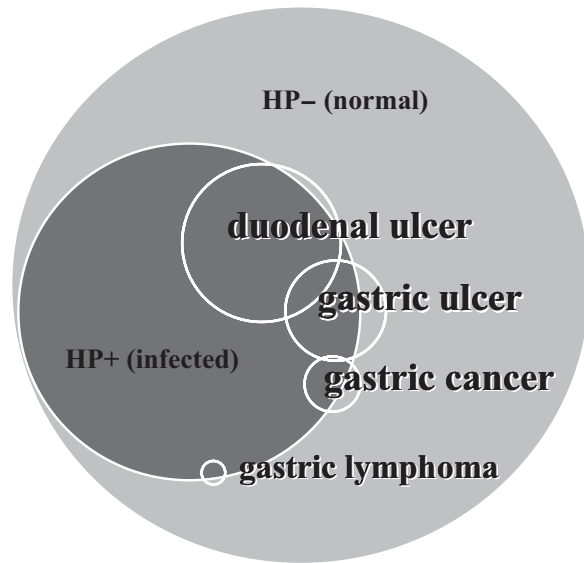
In cases of duodenal ulcer, but also in normal persons, we found islands of gastric metaplasia in the duodenal bulb. These islands make the proximal duodenum extremely susceptible to the effects of *H. pylori*. The high acid secretion noted in some duodenal ulcer patients is possible because gastritis only results in very superficial colonization of the acid-secreting corpus mucosa.

Contrast this with gastric ulcer patients, in whom acid secretion is lower because the gastritis is more widespread and the mucosal damage from gastritis is more severe. Gastric ulcer tends to occur at the junctional area between the antrum and the corpus, at locations of mixed intestinal and gastric-type mucosa. Histologically, the borders of duodenal ulcers and gastric ulcers look quite similar because they both tend to have this mixture of gastric (metaplasia) and intestinal (metaplasia) tissue types.

Some patients have a duodenal ulcer when they are young when they have high acid secretion, but suffer from gastric ulcer when they are older as worsening gastritis leads to lower acid secretion.

In the duodenal ulcer patient, there is usually far more neutrophil infiltration in the duodenum than in the stomach. It seems that the mucosa cannot perform both conflicting functions of a) withstanding acid attack and b) also allowing neutrophils to pass through to the lumen. Acid and digestive enzymes therefore penetrate the leaky mucosa and the peptic ulcer is created.

Changes in acid secretion, particularly high acid secretion, are well described in patients with duodenal ulcer. This occurs because gastrin secretion is slightly higher than average in patients with *H. pylori*. Normally, the antral G cell secretes gastrin, a short-lived hormone which stimulates the parietal cell to make acid. Gastric pH is sensed by the antral D cell, which produces somatostatin, a locally acting hormone, to turn off the gastrin. Unfortunately, when *H. pylori* causes gastritis, the inflammation destroys D cells so that there is less inhibition, more gastrin, and more acid. Patients with duodenal ulcer typically will secrete acid during the night when the stomach is empty be-



**Fig. 1** Venn Diagram showing disease associations with *H. pylori*.

cause they do not have an efficient negative feedback mechanism to turn off the acid.

### Disease Associations

As shown in Fig. 1, the disease associations of *H. pylori* and ulcers are well known. Gastric lymphoma is rather rare but it does show that at least some immunologic malignancies might be precipitated and maintained by the presence of *H. pylori*. There are even cases where a lymphoma outside the stomach, for instance in the tonsils or in the salivary gland, went into remission following treatment for *H. pylori* in the stomach.<sup>11</sup>

### Gastric Cancer

The gastric cancer link to *H. pylori* was first shown in the Eurogast study<sup>12</sup> in which gastric cancer incidence and *H. pylori* prevalence were compared within many countries. In that study, the highest rate for both *H. pylori* and cancer was in Yokote in northern Japan. Conversely, Minneapolis USA had both the lowest prevalence for stomach cancer and for *H. pylori*. The high incidence of gastric cancer in Japan, if due to *H. pylori*, may be entirely correctable. Uemura and colleagues have shown an apparent decline in cancer development in Japanese patients who have had eradication of their *H. pylori*.<sup>13</sup>

But not all persons with *H. pylori* have the same cancer risk. Dr. Uemura's group has also shown data whereby the cancer risk varied for different types of *H. pylori* related disease.<sup>14</sup> Importantly, if the patient had a duodenal ulcer, then the patient did not develop stomach cancer. This suggests that a normal or high

acid level protects from stomach cancer caused by *H. pylori*. Even though the *H. pylori* is *cagA* (cytotoxin associated gene) positive in duodenal ulcer, stomach cancer is rare. This shows that acid secretion is very important for the carcinogenic effect of *H. pylori*. With lesser acid secretion states – hyperplastic polyps, active gastric ulcer, non-ulcer dyspepsia – cancer risk may be inversely related to acid secretion. In Japan, the reason that stomach cancer is common may be the combined effects of *H. pylori*, low acidity, dietary interactions, and even genetic predisposition.

### Other Diseases

Other diseases have been suggested but not proven to be *H. pylori*-related. This issue causes an ongoing controversy.<sup>15</sup> Increased cardiovascular disease and also growth retardation have been associated with *H. pylori*.<sup>16</sup> These are difficult to separate from socioeconomic risk factors. Halitosis may be present in some patients, perhaps related to the achlorhydria which can occur allowing food to putrefy in the stomach. I believe 30% of chronic halitosis is caused by *H. pylori* but it is often impossible to separate *H. pylori* halitosis from other causes since all respond to the heavy antibiotic regimens used to treat *H. pylori*. Iron deficiency has been related to *H. pylori* in many studies so asymptomatic gastritis might be considered at the end of a negative workup for more serious causes of anaemia. Raynaud's disease, migraine headaches, Parkinson's disease, and idiopathic thrombocytopenic purpura are also receiving attention.<sup>15</sup> Regardless of the exact mechanism involved, *H. pylori* certainly does cause a chronic, lifelong stimulation of the immune system, so the potential exists for immune diseases to be initiated by *H. pylori* infection.<sup>15</sup>

### The VacA and CagA Toxins of *H. pylori*

To examine the pathogenesis of *H. pylori* disease in more detail, one must understand the *cagA* Pathogenicity Island (PAI). Whereas the *vacA* (vacuolating toxin) gene codes for a secreted toxin that causes vacuoles in epithelial cells, the Cag-PAI triggers interleukin-8 (IL-8) secretion, a neutrophil attractant. In addition, the Cag-PAI is a type IV bacterial secretion system. Type IV secretion systems were first identified in a bacterium called *Agrobacterium tumefaciens*.<sup>17</sup> This prototype organism is used in plant molecular biology to inject new genes into plants. Similarly, the type IV secretion system represented by *H. pylori*'s Cag-PAI injects CagA protein into the gastric mucosal cells. In strains with Cag-PAI, all aspects of *H. pylori*-associated disease appear to be more severe. Knowing this has allowed several groups to patent components of

this secretion system as potential vaccine targets. The two most likely antigens are CagA and CagE. CagE is an ATPase responsible for powering the Cag-PAI injection mechanism.<sup>18</sup> Thus, a vaccine or drug which could disable the CagE, might also cause CagA not to be secreted and thus would disable the harmful CagA effect.

Separate, but related to the Cag-PAI is VacA. VacA is a toxin that creates pores in the epithelial cells. These pores allow urea to pass out through the cells. To use the urea as an alkalinization mechanism, *H. pylori* has a urea-specific pore called UreI in its own cell wall that allows urea into the bacterial cell.<sup>19</sup> Because UreI is essential for colonization of the gastric mucosa it is yet another potential vaccine target.

### The *H. pylori* Genome

Tomb *et al* sequenced the *H. pylori* genome in 1997.<sup>20</sup> Its size of 1.7 megabases, or 1,600 genes, means that *H. pylori* is a rather simple organism with a genome only half the size of *Escherichia coli*.<sup>21</sup> This reflects the fact that *H. pylori* does not need a large complement of genes because the stomach mucosal environment is fairly constant and there is little competition from other bacteria. In fact the essential genes in *H. pylori* only number about 750, with the others being omitted in some strains.

Fifty-five percent of *H. pylori* genes have homologues in other life forms, but at least 23% are unique to *H. pylori*.<sup>22</sup> So this 23% of genes in *H. pylori* should be specific for survival in the stomach. By studying these “gastric” genes, we are certain to learn a lot about gastric mucosa. For example, we might better understand how organisms attach to the enteric mucosa or how *H. pylori* evades the mucosal immune system.

Gene arrays are now available that represent the complete *H. pylori* genome. Of course you can also obtain subsets of the human or mouse genome on a chip. This allows a typical experiment in which cells infected with *H. pylori* expressing Cag-PAI or a Cag-negative control strain of *H. pylori*. On the array, it is possible to see which genes of the mammalian genome are being activated or suppressed by the Cag-PAI.

With this strategy, we can identify factors in *H. pylori* and in humans, which determine the outcome of *H. pylori* disease. One can potentially identify strains of *H. pylori* that are less harmful, and possibly even “good”!

### Can *H. pylori* do Good?

#### Can Eradication of *H. pylori* be Bad?

There has arisen a controversy related to “good” vs. “bad” *H. pylori* as a result of a paper by Nyren *et al*

in Sweden entitled “Symptomatic Gastroesophageal Reflux as a Risk Factor of Esophageal Cancer”.<sup>23</sup> This article showed that acid reflux in the esophagus is a risk factor for adenocarcinoma in the esophagus. If the patients had complained of heartburn or reflux at least once a week the relative risk of adenocarcinoma in the esophagus was 7.7–10.8 times the control group without heartburn. This implied that factors which raised acid levels, even from low levels back up to normal, might increase esophageal adenocarcinoma risk. This paper alarmed gastroenterologists, particularly in the United States. They had noted an increased incidence of adenocarcinoma of the esophagus.<sup>24</sup> Was the decline in *H. pylori* partially responsible for the rising incidence of esophageal adenocarcinoma?

In an attempt to explain the relationships between *H. pylori*, acid secretion, and stomach cancer, the following hypothesis has been developed: If you acquire *H. pylori* at a very young age, the gastric mucosa is damaged. Damage is particularly severe if nutrition is poor. As a result of these adverse events, acid secretion is too low for peptic ulcer to occur, but cancer risk is increased. If you acquire *H. pylori* after the age of five years, then the corpus mucosa is spared the severe injury. Acid secretion is normal or high, and you are susceptible to ulcer disease, but less likely to develop stomach cancer.

We know that *H. pylori* is disappearing in Japan. High technology and attention to hygiene have largely protected young children from the infection. So there is less fecal-oral transmission of *H. pylori* in Japan.<sup>25</sup> However, declining *H. pylori* and improved nutrition are probably raising the acid secretion level of the whole Japanese population. Maybe this will cause more acid reflux disease and, subsequently, more adenocarcinoma of the esophagus. American gastroenterologists have suggested that reflux disease has increased in the USA because the prevalence of *H. pylori* has declined.<sup>26</sup>

So is there a downside to *H. pylori* eradication? Maybe the Cag-PAI strains protect us from reflux and from adenocarcinoma of the lower esophagus. But what is worse? The gastric cancer risk from *H. pylori* is at least 50 per 100,000 per year, but the esophageal risk of not having *H. pylori* is much lower, only 4.<sup>27</sup> So at the moment, there is a ten-fold benefit from *H. pylori* eradication. Of course if the patient has symptomatic heartburn, we can easily control this with a proton pump inhibitor, so this risk of acidity is probably low.

The future of *H. pylori* management might rely on a successful vaccine. There are many unique outer membrane proteins that can be used as vaccine targets in *H. pylori*. Ideally, we want the presence of the antigen in all strains of *H. pylori*. Although the essential genome of *H. pylori* is only about 750 genes, Covacci’s group

believe that the *Cag-PAI*-positive *H. pylori* is the most important to eradicate, so they have incorporated a *CagA* antigen in their vaccine.<sup>28</sup> The *UreI* is probably also a good vaccine candidate because it must be partly exposed on the surface of the *H. pylori*.

A typical vaccine experiment is usually first performed in mice. Covacci's group has a vaccine against neutrophil attracting protein (NAP), which gives considerable protection from *H. pylori*. They have also seen 80% protection with *CagA* and with whole organisms they see 90% protection. A new vaccine combining three antigens is as effective as the whole bacterium vaccine.<sup>28</sup>

At the present time, studies in healthy, normal persons with *H. pylori* are under way. However, it is likely to be at least ten more years before we can see a useful vaccine for *H. pylori*.

### Origins of *H. pylori*

Where does *H. pylori* come from? Recent data suggests that it followed humans out of Africa into Europe, and eventually to China about 20,000 years ago. Strains in Europe can be identified as type I compared with Japanese/Chinese strains, which are called type II.<sup>29</sup> But one of the questions is, "Are strains in Brazil and Venezuela European or Asian?" We now know that the strains of *H. pylori* in very remote South American Indian tribes actually resemble Asian strains.<sup>30</sup> This suggests that *H. pylori* has been migrating with humans over the past 30,000 years. Nowadays, most people in America have a Spanish or European strain, but probably that has just replaced the Asian strain.

### Transmission of *H. pylori*

We still do not know exactly how *H. pylori* is transmitted. One of the ways that we can study the epidemiology is with a swallowed string. One hour after swallowing the string, we pull the string out of the patient's mouth. Adhering to the lower end we now have culturable *H. pylori*. Recently we studied married couples that were both infected with *H. pylori*. By using AFLP techniques, we could show that strains from the wife were almost identical to the husband's strains. So it appears that some couples are behaving like a single culture medium with the same strain of *H. pylori* moving between them. Maybe *H. pylori* can be spread in families by kissing, but there is still a lot of controversy about his theory.

### Cost Effectiveness of *H. pylori* Eradication

Finally, let us consider the issue of whether it is cost effective to eradicate *H. pylori* in the whole popu-

lation. In this analysis, I will compare *H. pylori* to dandruff, which is a common scaly scalp condition. Both of these diseases are chronic inflammatory processes, often asymptomatic, with a known microbial agent. Dandruff is caused by a fungus and gastritis is caused by *H. pylori*. Reasons for therapy in both are cosmetic; i.e. white flakes in the scalp, or redness with bad histology in the stomach. But the cost of treatment is much higher for dandruff. Using over-the-counter medication, sufferers might spend USD\$15 a month, or USD\$1,500 over ten years. So USD\$1500 is the lifetime cost of treating dandruff, yet many people in the population choose to buy dandruff treatment.

If you ask these same people if they would like to take a treatment that could decrease cancer in the Japanese population and only cost USD\$100, they might perceive this as even better value than treating dandruff. On a cost effectiveness assessment, particularly in Japan, eradication of *H. pylori* seems advisable as soon as we obtain confirmatory evidence that *H. pylori* eradication prevents cancer. Policymakers will soon realize that it is very good value to eradicate *H. pylori* in Japan.

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