# Two decades of *Helicobacter pylori*: A review of the Fourth Western Pacific *Helicobacter* Congress

Carlo A Fallone MD[1], Naoki Chiba MD[2], Alison Buchan PhD[3], Bin Su PhD[4], Diane Taylor PhD[5]

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From March 3 to 6, 2002, *Helicobacter* enthusiasts gathered in Perth, Australia for the Fourth Western Pacific *Helicobacter* Congress to celebrate the 20th anniversary of the modern discovery of this organism by Barry Marshall and Robin Warren. The meeting included state-of-the-art lectures highlighting the breakthroughs that have occurred since the discovery of this bacterium. As well, advances from the forefront of current *Helicobacter pylori* research were presented, particularly in the realm of genomics and molecular biology. A symposium about vaccines and trends for future *H pylori* research completed this congress. The purpose of the present review is to summarize the highlights from this conference, emphasizing new advances.

**Key Words:** *Fourth Western Pacific* Helicobacter *Congress;* Helicobacter pylori

## *Helicobacter pylori* deux décennies plus tard : synthèse du 4e congrès du Pacifique-Ouest sur *Helicobacter*

**RÉSUMÉ :** Du 3 au 6 mars 2002, des passionnés d'*Helicobacter* se sont réunis à Perth, en Australie, en vue du 4e congrès du Pacifique-Ouest sur *Helicobacter* pour souligner le 20e anniversaire de la découverte du micro-organisme par Barry Marshall et Robin Warren. Des conférences à la fine pointe de l'actualité ont fait état des percées effectuées dans le domaine depuis la découverte de la bactérie. On y a présenté également les progrès réalisés à l'avant-garde de la recherche sur *Helicobacter pylori*, notamment en génomique et en biologie moléculaire. Enfin, un colloque sur la mise au point de vaccins contre la bactérie et les tendances de la recherche future sur le micro-organisme a complété le tout. Le présent article vise à présenter une synthèse des éléments saillants du congrès et à mettre en évidence les progrès réalisés dans le domaine.

[1]*Division of Gastroenterology, McGill University, Montreal, Quebec;* [2]*Division of Gastroenterology, McMaster University, Hamilton, Ontario;* [3]*Department of Physiology, University of British Columbia, Vancouver, British Columbia;* [4]*Department of Laboratory Medicine Pathology, University of Toronto, Toronto, Ontario;* [5]*Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta*
*Correspondence: Dr Carlo A Fallone, Division of Gastroenterology, Room R2.28, McGill University Health Center – Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec H3A 1A1. Telephone 514-843-1616, fax 514-843-1421, e-mail cfallone@lan1.molonc.mcgill.ca*
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## ANIMAL MODELS

Several animal models continue to be developed for the study of *Helicobacter pylori*-associated diseases. Bjorkhölm (USA) described a novel approach to identifying virulence-associated genes using the germ-free transgenic mouse and microarray technology. The Mongolian Gerbil model was used by Takshi (Japan) to determine that the *vac*A s2/m2 genotype is a very important virulence factor in the development of a gastric mucosal lesion. Using a mouse model, Rourke (USA) determined that the progression of mucosa-associated lymphoid tissue lymphoma depends on both bacterial virulence factors and how long the infection had been present. Interestingly, other *Helicobacter* species were found to colonize mouse models. Wadström (Sweden) found that 75% of eight well-known mouse strains were positive for *Helicobacter typhlonicus*, *Helicobacter rodentium*, *Helicobacter mesocricetorum* and *H pylori* using polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis and DNA sequencing.

## GENOMICS AND MOLECULAR STUDIES

Falkow (USA) presented an excellent overview of custom-designed gene chips to quantify changes in *H pylori* gene expression. Alterations in gene transcripts between different strains of the bacterium and in response to coculture with a human gastric cell line (AGS) were outlined. Custom-designed mouse and human gene chips (Stanford Genome Centre, USA) track how mammalian gene expression patterns are affected. The mouse model used BALB/C mice infected with the Sydney strain and the human model used AGS cells infected with a virulent strain of *H pylori*. The data from the human gene chip experiments identified the small GTPase, cdc42, as a major target of *H pylori*. Falkow then discussed how *H pylori* affected cell to cell adhesion molecules. A substrain of *H pylori* capable of attaching to the polarized epithelial cell line, MDCK, was isolated. The attachment sites of these bacteria correlated with the distribution of the tight junction protein, ZO-1.

In the same session, Trust (USA) discussed how designer drugs are generated using genomes. The genomes of 61 bacteria are available, and these data can be mined to identify bacteria-specific genes. A subset of essential genes can be determined, and targets for those genes developed. Screening of the *H pylori* genome unfortunately has not yet identified any obvious drug targets.

In a free paper session, the use of new genetic technologies was discussed. S Hjalmarsson (Sweden) dealt with pyro sequencing that gives fast, accurate sequence data on 20- to 25-base pair sections of genes. Further information on the technology can be found at www.pyrosequencing.com. Wadstrom (Sweden) discussed the use of protein chip technology to monitor changes in protein profiles. Blomstergren (Sweden) presented ongoing studies of the total genome analysis of three *H pylori* strains. His data so far indicate that the *cag*A gene is highly variable and that changes in the mRNA are translated into changes to the final protein structure. Wu (Taiwan) examined the effect of deleting the *flg*K gene encoding a flagellar protein homologous to the *Escherichia coli* hook-associated protein. Deletion had no effect on either adhesion or urease production. The bacteria colonized mice but at a lower density than wild-type bacteria. Buchan (Canada) discussed the use of gene arrays to dissect the differences between primary gastric epithelial cells and commonly used human gastric cell lines infected with *H pylori* on human gene expression. None of the three human gastric cell lines examined (AGS, NCI-N87 and MKN45) were found to be good models of the normal cells. Infection with *H pylori* modulated the gene expression and cellular location of proteins involved in cell to cell adhesion and intracellular vesicular trafficking pathways.

In a related session, Blaser (USA) dealt with the use of genetic screens (guanine to cytosine ratio, trinucleotide difference index) to identify variable regions in the *H pylori* genome. These techniques confirmed the existence of multiple regions of genetic variation that show geographical strain differentiation. In individuals infected with *H pylori*, the bacterium mutates over time. Coevolution of the *bab*A and *bab*B genes was documented in a study of infected individuals in Holland over a seven-year period.

The considerable genomic diversity among strains of *H pylori* was discussed by Taylor (Canada). This diversity has been demonstrated by various molecular typing techniques, direct sequencing and, recently, microarray analysis. Macrodiversity (diversity in gene order) probably occurs by inversion and/or transposition within plasticity zones of low guanine and cytosine content (35%) relative to the rest of the genome (39%). It could account for loss of the *cag*A pathogenicity island (PAI), resulting in the selection of a less virulent strain. Microdiversity in *H pylori* is highlighted by the considerable nucleotide divergence due to synonymous substitutions (15% to 21% for *H pylori* genes compared with 1% to 2% for genes from *Salmonella typhimurium* strains). In addition, at least 27 genes contain simple nucleotide repeats, including outer membrane proteins, lipoprotein synthesis enzymes and DNA restriction/modification systems, where frameshift mutations are easily generated by replication slippage. *H pylori* also lacks a methyl-directed mismatch DNA repair system, which could increase the frequency of mutation. Such genetic changes control membrane lipid composition and Lewis antigen synthesis genes, the expression of which is known to vary during the course of infection and likely optimize the survival of *H pylori* in the stomach.

## HOST BACTERIAL INTERACTIONS

Apart from changing itself, *H pylori* may alter and regulate its environment. Mobley (USA) discussed how this bacterium induces both subtle and dramatic changes in its environment, allowing the organism to persist, in most cases, for decades. These changes include the modulation of the acquired as well as innate immune responses, including alterations in cytokine levels. Cytoskeletal rearrangements in the epithelium are induced by injecting *cag*A into the

host cell via a type IV secretion system synthesized by the bacterium. The pH is modulated by the combination of bacterial urease activity and *vac*A-mediated urea permeation through the epithelium. Finally, gene expression patterns of nonparietal cells are altered by the presence of the bacterium. The response to colonization by *H pylori* is usually compatible with a lengthy host-parasite relationship; however, overt damage can result from this interaction. The mechanisms for a number of these host-parasite interactions are now better understood and offer targets for both therapeutic intervention and prevention by vaccination.

A free paper session was held on the host immune response. Mitchell (Australia) demonstrated that the cytokine response (interferon gamma in particular) to *H pylori* in subjects from developed and developing countries may differ. Thus, geography may add to the diversity encountered in all aspects of the host-bacterial interaction. Two interesting talks on the adhesion of *H pylori* to the host cells via toll-like receptors (TLR) presented contradictory results. Su (Canada) demonstrated that TLR4 might act as a receptor for *H pylori*. TLR4 RNA is expressed and upregulated by gastric epithelial cells in response to *H pylori* infection. Protein expression of TLR4 is also increased in this setting. In contrast, Bäckhed (Sweden), who compared TLR mRNA expression from different cell lines, was unable to demonstrate any TLR4 expression in primary gastric epithelial cells and concluded that TLRs are not involved in gastric mucosal recognition of *H pylori*.

## *H PYLORI* AND PEDIATRICS

Megraud (France) presented results of a multicentre European Study of over 500 children aged two to 17 years undergoing endoscopy. He confirmed the appropriateness of the $^{13}$carbon urea breath test as a diagnostic test in children (sensitivity 96%, specificity 95%). Serology was not as good, and urine antibody detection was poor. Stool antigen testing results, shown to be excellent in adults (Sheu, Taiwan), were pending in this European pediatric study.

Several studies from Aboriginal communities (known to have a high prevalence of *H pylori*) were also presented, including one from a Northern Manitoba Community (Song, USA), which demonstrated the presence of *H pylori* via PCR from saliva and nipple samples, supporting the theory of oral-oral transmission.

There were few treatment studies in the pediatric population, but Casswall (Sweden) presented results of a large study of 131 patients aged 10 to 21 years using a single daily dose regimen of lansoprazole, azithromycin and tinidazole for six days, achieving 93% compliance. The intention to treat eradication rate, however, was disappointing (63%).

## *H PYLORI* AND GASTRIC CANCER

Several advances have occurred in the realm of gastric cancer research as it relates to *H pylori* in recent years. Given the high rate of this disease in Asia and Europe, there are many scientific contributions from these continents. Although a specific symposium was dedicated to gastric cancer in Asia, the theme of gastric cancer kept reappearing throughout much of the meeting. One of the most important recent developments has been the results of a study by Uemura et al (1), who demonstrated that gastric cancer was associated with *H pylori*; none of the uninfected patients developed cancer, whereas all of the patients who developed gastric cancer were infected with *H pylori*. In addition, those who were infected but whose *H pylori* was eradicated did not develop gastric cancer, although the duration of follow-up in this subgroup of patients was much smaller. A further advancement was the development of an excellent Mongolian gerbil model used to study the effects of *p53* gene expression in the development of gastric cancer. In addition, Sipponen (Finland) described a panel of blood tests, incorporating several markers of atrophic gastritis, including *H pylori* serology, pepsinogen ratios and gastrin 17 levels. Computer-calculated risks of having atrophic gastritis using these results demonstrated a good correlation with the histological diagnosis of atrophic gastritis, with a sensitivity and specificity of 80% to 90%. He concluded that this test could perhaps be used to detect those who may be at higher risk of subsequently developing gastric cancer.

In a free paper session, Van Doorn (the Netherlands) identified that, in *H pylori*-infected Portuguese patients, both *vac*A and *cag*A genotypes were associated with an increased risk of gastric carcinoma (odds ratio [OR] 6.7 to 17) and that patients with interleukin (IL)-1 polymorphisms such as IL-1β-511 T carriers and IL-1RN*2 also had an increased risk (smaller OR of 3.3). However, what was particularly striking was that those with both virulent *H pylori* genotypes in association with human IL-1 polymorphisms had a dramatically increased risk of gastric cancer, suggesting a synergistic interaction between bacterial virulence and host genetic susceptibility (OR as high as 108, 95% CI 10 to 1148 if *vac*A s1 and IL-1 polymorphism existed). This may partly explain why only some patients develop gastric cancer.

There was interest in elucidating the role of *cag*A+ strains as a marker of increased gastric cancer risk. PAI leads to nuclear factor kappaB and IL-8 secretion, which may play a central role in host response to *H pylori* infection. *CagA* is one of the markers of the PAI. Wu (United Kingdom) reported that patients who were seropositive for *cag*A had a significantly increased risk of distal gastric cancer (OR 2.1, 95% CI 1.1 to 9.3) but no influence on junctional (gastric cardia and esophageal adenocarcinoma) cancer. Previous studies have suggested that patients infected with a *cag*A-positive strain may be protected against junctional cancers. At this meeting, a recurring warning was that the traditional *cag*A ELISA serological tests may not be sufficiently sensitive or specific enough and that *cag*A determination through PCR or immunoblotting was preferred (Shimoyama, Japan; Engstrand, Sweden). Engstrand presented data on 298 Swedish patients with gastric carcinoma and found that 76% were positive for *H pylori* by ELISA, whereas 88% of those who were negative on ELISA were positive for *cag*A by

immunoblot. This indicates that, unless researchers also look for other markers of *H pylori*, the gastric cancer risk associated with *H pylori* could be underestimated.

## *H PYLORI* AND ASSOCIATION WITH OTHER CLINICAL DISEASES

*H pylori* is associated with peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer. Controversial associations include functional dyspepsia, gastroesophageal reflux disease (GERD) and nonsteroidal anti-inflammatory drug (NSAID)-induced ulcer disease.

The role of *H pylori* in predicting endoscopic findings in patients with uninvestigated dyspepsia from the Canadian Adult Dyspepsia Empiric Therapy – Prompt Endoscopy (CADET-PE) study was presented by Chiba (Canada). In the 1013 patients with available *H pylori* status, *H pylori* prevalence was 30% and increased with age. Overall, clinically significant findings were observed in 58% of all patients. Gastric and duodenal ulcers were rare (3% or less for each), but both were more common if *H pylori* was present (5.6% versus 2.0% and 6.6% versus 1.3%, respectively; each $P<0.002$). Erosive esophagitis was the most common overall finding. The prevalence of esophagitis was less common in *H pylori*-positive (36%) than in *H pylori*-negative (46%) patients ($P<0.002$). The results of this study suggest that, while endoscopic findings are common, the vast majority would be appropriately treated with empirical acid-suppressive therapy and prompt endoscopy would not necessarily alter initial management.

While it is well accepted that *H pylori* and NSAIDs are the most important causes of duodenal ulcer disease, it is also recognized that there is an increasing population that appears to be negative for *H pylori* through traditional testing of gastric biopsies. An interesting study demonstrated that *H pylori* was present in the duodenum of 6.8% of patients who did not have *H pylori* in their gastric specimens. This report suggests that additional duodenal biopsies may help decrease the prevalence of apparently *H pylori*-negative ulcer subjects (Kullavanijaya, Thailand).

Hawkey et al (2) found that those who were infected with *H pylori* experienced greater healing of NSAID-induced gastric ulcer. However, Chan et al (3,4) showed that the eradication of *H pylori* reduced the risk of ulcer development. With these somewhat discrepant results and observations, Huang et al (5) reported a recent meta-analysis that demonstrated that *H pylori* increased the risk of NSAID-associated ulcer and bleeding. This was in keeping with data presented at this meeting of a single centre experience of 125 patients in Thailand (Mahachai) in which upper gastrointestinal bleeding was seen more frequently in NSAID users who were co-infected with *H pylori* than in those who were not co-infected with *H pylori*. Although *H pylori* and NSAIDs are the most important risk factors for ulcer and bleeding, 27% of bleeding subjects in one study (Ootani, Japan) had neither risk factor.

The effect of *H pylori* on GERD is still controversial, with some studies suggesting an increased incidence of GERD after *H pylori* eradication (6,7) and more recent publications (8,9) suggesting that eradication does not worsen GERD. Fallone (Canada) presented the results of an interim analysis demonstrating that GERD patients who were negative for *H pylori* had more severe GERD, as determined by the Spechler Gastrointestinal Reflux Disease Activity Index, and used proton pump inhibitors more often than infected patients. Other validated GERD severity scores, including questionnaires and 24 h pHmetry, demonstrated similar trends. These results suggest that *H pylori* infection results in less severe GERD and is in keeping with the CADET-PE data showing that *H pylori*-positive patients had a lower prevalence of endoscopic esophagitis than uninfected patients.

## TREATMENT AND ANTIBIOTIC RESISTANCE

Koga (Japan), presented a novel finding using probiotics to eradicate *H pylori*. He found that *Lactobacillus gasseri* OLL2716 (LG21) eradicated *H pylori* in mice. In humans, it was shown to decrease urea breath test $^{13}$carbon levels at 24 weeks. In an in vitro study, Vilaichone (Thailand) found that *Lactobacillus acidophilus* had an inhibitory effect on *H pylori* in 87% of patients tested. These agents certainly require further study, but it is very exciting to see an agent as innocuous as yogourt work in the treatment of *H pylori* infection.

A key predictor of eradication failure is antibiotic resistance. Clancy (Australia) showed that one-third to one-half of those who had failed to respond to therapy were infected with *H pylori* that had been shown to be resistant to metronidazole or clarithromycin when these antibiotics were used as part of triple therapy. Host factors also played a part in treatment failure, including longer symptom duration, regular alcohol ingestion and low levels of IL-4 secretion. A high prevalence of clarithromycin resistance (40%) was reported in an urban area in central Italy (Toracchio). This rate is much higher than that cited in Canada and the United States (1% to 5%), or in other parts of Europe (1% to 25%).

With regard to the causes of metronidazole resistance, Taylor (Canada) demonstrated that mutations in the *frx* gene only occurred in metronidazole-resistant strains, although not as frequently as mutations of the *rdx*A gene encoding a reduced nicotinamide adenine dinucleotide phosphate nitroreductase. Often, double *rdx*A, *frx*A mutations were found. Denaturing high performance liquid chromatography (dHPLC) is a new method for detecting metronidazole-resistant mutations and was compared with other PCR-based methods (Tuazon, USA). Although dHPLC can detect mutations at A2142 or A2143 in 23S ribosomal RNA genes, it cannot identify which position is mutated. Nevertheless, the results are rapidly available in a couple of hours, and dHPLC has great potential as a screening technique, although it has yet to be used directly with biopsy material.

A Belgium team (Lamy) reported that their routine clinical strategy since 1988 has been to perform *H pylori* anti-

bacterial susceptibility testing and provide these results to general practitioners before eradication therapy is given. Even with their strategy, imidazole resistance increased from 16.9% to 20.7%, clarithromycin resistance increased from 6.7% to 14% and multiresistance increased from 1.5% to 2.8%. However, their resistance rates were lower than for the rest of Belgium, where imidazole resistance ranges from 20% to 40%, macrolide resistance from 3% to 25% and multiresistance from 5% to 10%. They believed that their practice influenced general practitioners' choices of eradication therapy – an important consideration because the overall rates of resistance are gradually rising.

## VACCINES

A vaccine against *H pylori* has been sought for many years. In a symposium dedicated to the topic, Tetlin (USA) first described how the Institute for Genomic Research makes use of genomic data for the development of novel vaccine candidates. There are currently 61 complete microbial genomes available (see www.tigr.org), allowing comparisons and alignments to be performed at the DNA and protein levels. Surface-exposed lipoproteins have been used as vaccine targets for certain organisms such as *Neisseria meningitidis*. Rapuoli (Italy) subsequently discussed the *H pylori* vaccine. Because of the association of *H pylori* virulence factors with peptic ulcer and gastric cancer, Chiron (USA) developed a vaccine using *cag*A, *vac*A (the vacuolating cytotoxin) and NAP (neutrophil activating protein) components of *H pylori*. This is different from other vaccines, which are based on the urease enzyme (Aebisher, Germany). In mice, the Chiron vaccine eradicated *H pylori* in 80% of animals and prevented reinfection. Malfertheiner (Germany) described the results of a phase I clinical trial with this vaccine on healthy German volunteers. The Chiron *cag*A, *vac*A, NAP vaccine elicited both a significant immunoglobulin G antibody response and a cellular immune response in terms of cytokine profile. A mild skin reaction was noted, which was also present in controls and is related to the use of aluminum hydroxide as the adjuvant. Occasional headache and slight fever, but no severe adverse effects, were also noted. Results of additional trials for eradication and protection from *H pylori* infection in humans are eagerly awaited.

Finally, Graham (USA) discussed the pharmacoeconomics of an *H pylori* vaccine. Although in developed countries the incidence of *H pylori* is decreasing, the high prevalence in developing countries, the distinct association with the development of gastric cancer and the increase in antibiotic-resistant strains are likely to make an *H pylori* vaccine a useful adjunct to antibiotic-based therapies for eradication and protection.

## CONCLUDING REMARKS

One highlight of the meeting was a lively debate on the future of *H pylori* research. Specifically, the motion that "the *H pylori* bubble had burst" was defended by Graham, Blaser and Mobley (USA), who agreed that the pharmaceutical industry had substantially reduced, if not eliminated, funding for *H pylori* research and that interest in the field was at an all time low because the pharmaceutical industry had mistakenly concluded that the most important areas had already been studied. On the other hand, Lee (Australia), Falkow (USA) and Forman (United Kingdom) opposed this motion. They argued that 40% of the world population was still infected by this gastric carcinogen and as indicated by Hu (China) earlier in the conference, *H pylori* remains a major problem in areas such as China and India. The pharmaceutical industry should realize that there are markets other than the Western world. In addition, this organism is a good organism to study for the purpose of advancing science in general, and for the study of the epidemiology, transmission and pathogenesis of gastric cancer.

This conference demonstrated that many questions about *H pylori* remain unanswered. Appropriately, it was Barry Marshall (Australia), the man who started it all, who summarized it best, when he stated that many lessons remained to be learned about the organism, which in turn can teach us an enormous amount about the human body.

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