Journal of Cellular Biochemistry 102:264–273 (2007)

PROSPECTS

Helicobacter pylori as a Class I Carcinogen: Physiopathology and Management Strategies

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Abstract The gram-negative bacterium *Helicobacter pylori* is known as a persistent colonizer of the human stomach, and probably less known is that it is also involved in extraintestinal diseases. Public awareness of its contribution in the development of gastric cancer is less than 15 years old. The efficacy of the current therapies based on antibiotics against *H. pylori* has been limited by difficulties such as antibiotic resistance and recurrence. As a consequence, the development of promising vaccines was prompted as the best preventive measure. Unfortunately, so far vaccines failed the transition from animal models to human trials. This keynote presentation is to provide a bird's eye view of *H. pylori*-related gastric diseases, including gastric cancer, with a synthesis of the molecular mechanisms involved, and an exhaustive presentation and discussion of the current therapeutic guidelines and future strategies for prevention or therapy. J. Cell. Biochem. 102: 264–273, 2007.

Key words: *H. pylori*; gastric cancer; prevention; antibiotics; vaccines

Helicobacter pylori, a common pathogen of humans, is a gram-negative microaerophilic bacterium that chronically infects gastric epithelial cell surfaces and the overlying mucin layer. It is a slow-growing bacterium with multiple, sheathed flagella. Like the genera *Campylobacter*, it is in the class V or *Epsilonproteobacteria*, but the latter is a spirally curved cell with a single polar flagellum at one or both ends. It shows helical, curved, or straight cells with rounded ends, and measures 0.2–1.2 μm × 1.5–10 μm [Garrity et al., 2005] (Fig. 1A–C). It is most likely spread from person to person through fecal–oral or oral–oral routes. Possible environmental reservoirs such as contaminated water sources, as well as iatrogenic spread through contaminated endoscopes have been documented. Support for the person-to-person transmission through close contact and exposure to vomit comes from evidence of clustering within families and from reports of higher than expected prevalence in residents of custodial institutions and nursing homes. An Italian pathologist of the University of Turin, Giulio Bizzozero, observed for the first time spiral bacteria in the stomach of dogs [Bizzozero, 1892], which were probably *Helicobacter felis* (present in normal gastric flora in cats and dogs and used today as model of *H. pylori* infection in murine models); however, his studies have been dismissed because the stomach was thought to be sterile and these bacteria were considered consequence of post-mortem contamination. Finally, in 1982, Robin Warren, a pathologist and Barry Marshall, a young internist in Perth, Western Australia are the first to report the cultivation of this microorganism from bioptic samples of gastric mucosa, called initially *Campylobacter pyloridis* [Marshall and Warren, 1984] and later

Grant sponsor: Sbarro Health Research Organization; Grant sponsor: National Institutes of Health.

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Received 16 March 2007; Accepted 19 March 2007
DOI 10.1002/jcb.21375

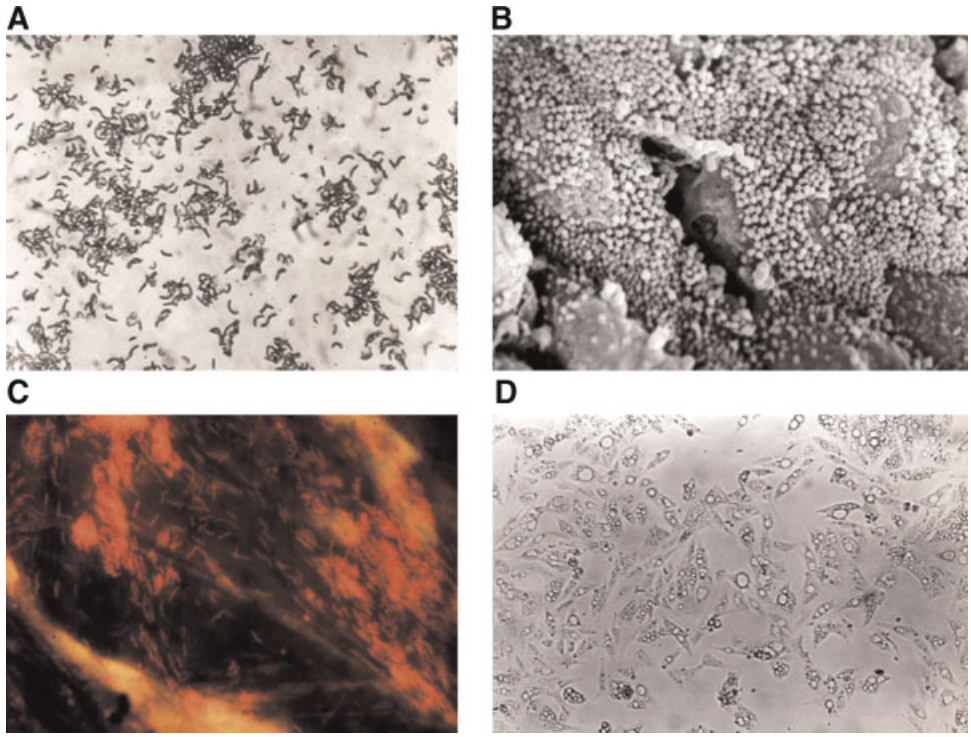

Fig. 1. **A**: In this picture, colonies with classic *H. pylori* morphology are shown. Gram-stained slide examined and photographed with a 100× immersion oil objective. Bright field microscope. **B**: Scanning electron microscopy indicates some spiral-shaped and several coccoid *H. pylori* bacteria adhering to gastric mucosa cells. The older cultures of *H. pylori* adopt a spherical shape, resembling cocci, and lack growth. **C**: A smear of gastric mucosa was flame-fixed and stained with acridine orange. The intensely stained bacteria and cores indicate the presence of significant amounts of RNA. UV microscopy. **D**: The majority of *H. pylori* strains express a 95-kD VacA, a secreted vacuolating cytotoxin, which contributes to gastric colonization [Luzzi et al., 1993] and interacts directly with immune cells. In particular, VacA alters the function of T lymphocytes, B cells, macrophages, and mast cells [Suerbaum and Michetti, 2002; Algood and Cover, 2006]. Here the vacuolating effect is shown on human cells in culture. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

H. pylori (Hp unlike *Campylobacter* cannot reduce nitrate and is urease positive). Thanks to this discovery they received the Nobel Prize in Physiology/Medicine in 2005. They sustained that Hp is not a harmless commensal but a pathogen that fulfills all four Koch's postulates. Today Hp is much more studied but many objections about these concepts are still present. As a matter of fact, *H. pylori* may also have a role in reducing the risk of gastroesophageal reflux disease and esophageal adenocarcinoma [Ye et al., 2004]. However, allowing infection with *H. pylori*, a class I carcinogen [IARC Working Group, 1994], to persist is improbable to be the ideal strategy for the prevention or therapy of gastroesophageal reflux.

H. pylori is the first microorganism for which the genomes of two different strains, the strain 26695 [Tomb et al., 1997], obtained from a patient in the United Kingdom with superficial gastritis and the strain J99 [Alm et al., 1999], obtained from a patient with duodenal ulcer disease, have been fully sequenced. Both have a circular genome, the former has a chromosome size of 1,667,867 base pairs (bp), an average G + C content of 39% and 1,590 predicted coding sequences and the latter has 95 fewer genes with 1,643,831 bp. Despite this, most of the information available for therapy strategies (vaccine candidates) was obtained in the pregenomic era, probably because of the lack of immunological correlates that may be predictive of immunity, and the lack of easy assays to test protection in vivo.

H. pylori may provoke chronic gastritis, ulcer disease (more than 90% of duodenal ulcers and up to 80% of gastric ulcers), atrophic gastritis, gastric cancer, and mucosal-associated-lymphoid-type (MALT) lymphoma and non-Hodgkin's lymphoma of the stomach [Hessey et al., 1990; Marwick, 1990; Stolte, 1992; IARC Working Group, 1994; Suerbaum

and Michetti, 2002; Vogiatzi et al., 2007]. Recently Lugli et al. [2007] comparing Napoleon's clinical and familiar history with clinicopathologic data from 135 gastric cancer patients, evaluated the numerous hypotheses on the cause of Napoleon Bonaparte's death (hereditary gastric cancer, arsenic poisoning, and inappropriate medical treatment), and suggested that Napoleon's main risk factor might have been *H. pylori* infection, associated to a polymorphism in the interleukin-1β (*IL-1β*) gene, which enhances the risk of stomach cancer. The role of *H. pylori* in non-ulcer dyspepsia remains unclear. Moreover, this bacterium has now been implicated even in extragastric diseases, including idiopathic thrombocytopenic purpura (ITP), iron deficiency anemia, chronic urticaria, ischemic heart disease, and others [Takahashi et al., 2004; Rad et al., 2006; Malfertheiner et al., 2007].

Hp infection prevalence is from 20 to 50% in middle-aged adults in industrialized countries while it reaches over 80% in some developing countries [Suerbaum and Michetti, 2002; Frenck and Clemens, 2003]. Although chronic *H. pylori* infection is the strongest known risk factor for development of gastric adenocarcinomas, only a small proportion of infected individuals will ever develop tumors. However, the risk of stomach cancer is twice as high for infected individuals [Huang et al., 2003]. The reason why some individuals remain *H. pylori* infected for life but without any symptoms while others develop severe diseases remains a topic to be clarified. The severity, progression, and consequences of *H. pylori* infection have been shown to depend on multifactorial interactions among host immunologic and physiologic factors, bacterial virulence determinants, and environmental influences modulating the host response.

MECHANISMS OF DISEASE, PREVENTION AND MODELS OF INFECTION

The *H. pylori* genome codes for about 1,500 proteins. Many Hp factors interact directly with immune cells, such as the cytotoxin-associated antigen CagA, the secreted vacuolating cytotoxin VacA (Fig. 1D) [Luzzi et al., 1993], the neutophil-activating protein (NapA), arginase, urease, Hsp60, SabA, HcpA, and a proinflammatory peptide named Hp (2–20) [Algood and Cover, 2006].

Many *H. pylori* strains harbor a 37-kb DNA chromosomal region called "*cag* pathogenicity island" or *cag*-pathogenicity island (PAI) containing 29 genes. Several of these genes are probably involved in the secretory machinery that translocates CagA (the product of the terminal gene in the island), a 120-kD protein, into the cytoplasm of gastric epithelial cells. After intracellular delivery CagA is phosphorylated by members of the Src family of kinases. Subsequently phosphor-CagA activates a eukaryotic phosphatase (SHP-2) leading to cellular morphological alterations [Suerbaum and Michetti, 2002]. Moreover, it has been observed that gastric cancer and peptic ulcer patients are infected more frequently by *cag*-PAI positive strains than by *cag*-PAI negative strains [Blaser, 2005] compared to asymptomatic patients.

Unlike the *cag* island, *vacA* gene is always present, whereas the VacA protein is produced by only 50–60% of the strains. VacA inhibits the activity of nuclear factor of activated T cells (NFAT), a transcription factor that regulates inmmune responses, in Jurkat T cells, resulting in decreasing IL-2 production and G1/S cell cycle arrest. *H. pylori* interfere with the prelysosomal processing of tetanus toxin in Epstein Barr virus-transformed B cells inhibiting the Ii-dependent pathway of antigen presentation. In addition, *H. pylori* strains expressing VacA interrupt phagosome maturation in macrophages by recruiting and retaining TACO (coronin 1) protein. VacA can also induce mast cell chemotaxis and stimulate mast cell expression of multiple proinflammatory cytokines, including IL-1, TNF, IL-6, IL-13, and IL-10 [Algood and Cover, 2006].

H. pylori null mutant strains with lack of the enzyme urease or flagella are unable to colonize the gastric mucosa in animal models. *H. pylori* urease is a hexameric protein of 550 kDa consisting of two subunits, UreA (~30 kDa) and UreB (~62 kDa), of which the B subunit (urease B) may be useful to induce immunity [Hatzifoti et al., 2006].

Several outer membrane proteins such as BabA, SabA, AlpA, AlpB, and HopZ, can mediate bacterial adherence to gastric epithelial cells. Among them, BabA protein of 78-kD, encoded by the gene *babA2*, is the best characterized adhesin to gastric epithelial cells, and binds to the fucosylated Lewis B blood-group antigen [Ilver et al., 1998]. The presence of

babA2 is associated with *cagA* and *vacA* s1 alleles and strains that possess all three genes determine the highest risk for gastric cancer [Gerhard et al., 1999].

Among the animal models used for investigating *H. pylori* infection were mice, Mongolian gerbils, guinea pigs, rats, ferrets, beagle dogs, cats, gnotobiotic piglets, and non-human primates [Chen et al., 1992; Kusters et al., 2006]. However, the most popular experimental model of *H. pylori* pathogenesis is the Mongolian gerbil model because the infection of these animals results in the development of gastric mucosal ulceration and cancer [Ogura et al., 2000]. Mouse models were suitable for vaccination experiments against *H. pylori* because the immunoproteomes expressed in mice were similar to humans and they showed stable colonization [Marchetti et al., 1995].

Knowledge of genome sequences (*H. pylori* and human), measurable phenotypes (CagA phosphorylation) and animal models can help to prevent gastric diseases and improve health for all. Host genetic factors, such as *IL-1* gene polymorphisms, in particular of *IL-1B* which encodes IL-1β cytokine and *IL-RN* encoding the IL-1 receptor antagonist, may interact in the complex process of gastric carcinogenesis [Palli et al., 2005] and people with high *IL-1β* expression and CagA strains clearly benefit from *H. pylori* eradication and by reduced cancer risk [Kusters et al., 2006]. In fact, eradication of *H. pylori* decreases the risk of gastric cancer in infected persons without precancerous lesions, providing an early target to halt gastric carcinogenesis [Vogiatzi et al., 2007]. Moreover, gastric cancer risk is the sum of the polymorphic nature of the bacteria in the host, the host genotype, and environmental exposures. Poor living conditions and hygiene are known as risk factors for *H. pylori* infection and *H. pylori*-associated diseases. Diets low in vegetables, fibers and fruits, and high in salt-preserved foods increase the risk of gastric adenocarcinoma [Vogiatzi et al., 2007]. Under this light the Mediterranean diet is another measure of prevention against stomach cancer.

DIAGNOSTIC TOOLS

Patients with active gastric or duodenal ulcers or documented history of ulcers, early gastric cancer, and low-grade gastric B-cell MALT lymphoma should be tested for *H. pylori* and if found to be infected, they should be treated. Retesting after treatment may be prudent for patients with bleeding or otherwise complicated peptic ulcer disease. To date, there has been no conclusive evidence that treatment of *H. pylori* infection in patients with non-ulcer dyspepsia is warranted and treatment recommendations for children have not been formulated.

The diagnosis of *H. pylori* is based on non-invasive methods or invasive, which is the endoscopic biopsy of the gastric mucosa. The choice of the appropriate test depends on the clinical setting. Non-invasive tests include the urea breath test, serologic tests, and stool antigen assays [Gisbert and Abraira, 2006; Kusters et al., 2006].

In the urea breath test, the patient is given either 13C- or 14C-labeled urea to drink. *H. pylori* metabolize the urea rapidly, and the labeled carbon is absorbed. This labeled carbon can then be measured as CO_2 in the patient's expired breath to determining whether Hp is present. This method is indicated for the initial diagnosis of the infection and for follow-up of eradication therapy. It is the best available non-invasive test in children but with higher false-positive rates in infants and children younger than 6 years compared with school-age children and adolescents.

Serological tests measuring specific *H. pylori* IgG antibodies can determine if a person has been infected. These diagnostic tests are simple, accurate, and widely used for the diagnosis in adult patients before treatment.

The stool antigen assays represent an alternative solution to the urea breath test with similar high sensitivity and specificity and are suitable even in young children.

The patients which shown symptoms, such as anemia, gastrointestinal bleeding, weight loss, or have more than 50 years of age should undergo esophagogastroduodenal endoscopy for the diagnosis of *H. pylori* infection and any associated pathology which may be already present. During endoscopy the test of first choice is a urease test on an antral-biopsy specimen, a rapid colorimetric test based on the ability of *H. pylori* to produce urease. When the second-line therapy fails, it is recommended to culture *H. pylori* with antibiotic-sensitivity tests accordingly to the guidelines of the National Committee for

Clinical Laboratory Standards [Suerbaum and Michetti, 2002].

THERAPEUTIC APPROACHES

The current therapy is complex and increase of bacterial resistance to current antimicrobials and treatment failure has called for new compounds or regimens. Here we report the more used antimicrobial agents and the challenges for vaccine development.

CONVENTIONAL TREATMENTS

Animal studies have shown that early eradication of *H. pylori* infection prevents efficiently *H. pylori*-related gastric carcinogenesis. However, the available data from human studies show that *H. pylori* eradication does not completely prevent gastric cancer and that it might be useful only in patients without atrophic gastritis or intestinal metaplasia at baseline.

Killing *H. pylori* is difficult due to antibiotic resistance and to its peculiar microenvironment, however much work has been done. The *H. pylori* treatment regimens approved by Food and Drug Administration (FDA) for *H. pylori* eradication are summarized in Table I (http://www.cdc.gov/ulcer/keytocure.htm; Drugs@ FDA). The standard treatment is a triple therapy with a proton pump inhibitor such as omeprazole, and two antibiotics such as amoxicillin, clarithromycin (Biaxin), or a nitroimidazole (metronidazole (Flagyl) or tinidazole). The proton pump inhibitors (omeprazole (Prilosec), lansoprazole (Prevacid), rabeprazole, pantoprazole (Protonix), esomeprazole) suppress acid production by halting the mechanism that pumps the H+ into the stomach, while H2 blockers (cimetidine, ranitidine (Zantac), famotidine, nizatidine) work by blocking histamine, which stimulates acid secretion; these drugs have been prescribed alone for years as treatments for ulcers. But used alone, they do not eradicate *H. pylori* and therefore do not cure *H. pylori*-related ulcers. Bismuth subsalicylate, a component of Pepto-Bismol, is used to protect the stomach lining from acid. Expected eradication success rates of the FDA-approved regimens range from 61 to 94% depending on the regimen used. Overall, triple therapy regimens have shown better eradication rates than dual therapy. Longer length of treatment (14 days vs. 10 days) results in better eradication rates.

According to the official recommendations of European Helicobacter Study Group (EHSG) approved by the third Maastricht Consensus conference [Malfertheiner et al., 2007], the

TABLE I. FDA-Approved Treatment Strategies for *H. pylori* Eradication

Drug regimen	Dosage	Time of administration
Esomeprazole magnesium	40 mg once daily	
Plus amoxicillin	1 g twice daily	
Plus clarithromycin (Biaxin)	500 mg twice daily	For 10 days
Rabeprazole sodium	20 mg twice daily	
Plus amoxicillin	1 g twice daily	
Plus clarithromycin	500 mg twice daily	For 7 days
Omeprazole (Prilosec)	40 mg daily	
Plus clarithromycin	500 mg three times daily	For 2 weeks
And then omeprazole	20 mg daily	For other 2 weeks
Omeprazole	20 mg twice daily	
Plus clarithromycin	500 mg twice daily	
Plus amoxicillin	1 g twice daily	For 10 days
Lansoprazole (Prevacid)	30 mg twice daily	
Plus amoxicillin	1 g twice daily	
Plus clarithromycin	500 mg twice or three times daily	For 10 days
Lansoprazole	30 mg three times daily	
Plus amoxicillin	1 g three times daily	For 2 weeks[a]
Bismuth subsalicylate (Pepto-Bismol)	525 mg four times daily	
Plus metronidazole (Flagyl)	250 mg four times daily	
Plus tetracycline[b]	500 mg four times daily	For 2 weeks
H2 receptor antagonist		For 4 weeks
Ranitidine bismuth citrate	400 mg twice daily	
Plus clarithromycin	500 mg twice or three times daily	For 2 weeks
And then ranitidine bismuth citrate	400 mg twice daily	For other 2 weeks

[a]This dual therapy regimen has restrictive labeling. It is indicated for patients who are either allergic or intolerant to clarithromycin or for infections with known or suspected resistance to clarithromycin.
[b]Although not FDA approved, amoxicillin has been substituted for tetracycline for patients for whom tetracycline is not recommended. The tetracycline should not to be used for children younger than 12 years.

eradication of *H. pylori* infection is indicated in patients with gastro-duodenal pathologies, such as peptic ulcer disease, and low-grade MALT lymphoma, atrophic gastritis, first-degree relatives of gastric cancer patients, unexplained iron deficiency anemia, and chronic ITP. If other causes are excluded, recurrent abdominal pain in children is an indication for testing and treating strategy. The eradication of *H. pylori* infection does not cause or exacerbate gastro-oesophageal reflux disease, and it may prevent peptic ulcer in patients who are naive non-steroidal anti-inflammatory drugs (NSAIDS) users. Triple therapy using a proton pump inhibitor with amoxicillin and clarithromycin or metronidazole given twice daily remains the recommended first choice therapy. Bismuth containing quadruple therapy, if available, is also a first choice treatment option [Malfertheiner et al., 2007]; although it is more effective against *H. pylori* than triple therapy, it is frequently not prescribed because of the more complex dosing schedule and side effects.

Antibiotics-based *H. pylori* eradication treatment is quite effective. However, it is expensive and may cause mild side effects such as nausea, vomiting, diarrhea, a temporary grayish-black discoloration of the stool (associated with Pepto-Bismol), a bitter or metallic taste in the mouth (caused by clarithromycin), dizziness, headache, and yeast infections in women, and of course antibiotic resistance.

Resistance to nitroimidazoles (metronidazole, tinidazole) is the most common form of antimicrobial resistance to *H. pylori*. In industrialized countries about 35% of *H. pylori* strains are resistant to nitroimidazoles, whereas in developing countries this value can be as high as 95%. A possible explanation is the frequent use of these drugs for the therapy of parasitic-related diseases, while in developed countries they are limited to the treatment of gynecological and dental infections [Gerrits et al., 2006]. Metronidazole resistance among *H. pylori* strains is caused primarily by mutations in nitroreductase genes, mainly including oxygen-insensitive nitroreductase, *rdxA* gene, and flavin oxidoreductase, *frxA* gene that interfere with the intracellular activation of nitroimidazoles. Moreover, it has been shown that development of metronidazole resistance in *H. pylori* requires inactivation of *rdxA* alone or of both *rdxA* and *frxA*, depending on bacterial genotype, but rarely, if ever, inactivation of *frxA* alone, and that *H. pylori* strains differ in regulation of nitroreductase gene expression [Jeong et al., 2001]. Resistance to macrolides (clarithromycin, erythromycin) is also present and is caused by mutations in 23S rRNA genes; in the late 1990s its prevalence ranged from 10% of the Western countries to 50% in developing countries [Gerrits et al., 2006].

CURRENT STATUS OF VACCINES AND EFFICACY

The effort of developing a vaccine against *H. pylori* infection is justified by many reasons: the high antibiotic resistance, the problems of patient compliance, the possible recurrence or re-infection in areas with high prevalence, and especially the fact that many individuals at risk to develop severe complications of *H. pylori* infection, such as atrophic gastritis and stomach cancer, are asymptomatic.

An effective vaccine, which antigen should be shared by all *H. pylori* isolates, induce potent specific immune response, lack intrinsic toxicity, and even cause tumor regression, would be a desirable way to control *H. pylori*-induced gastric diseases. Several protective antigens have been identified, including urease, VacA, CagA, and other PAI elements, two heat-shock proteins (HspA and HspB), catalase, and chemotaxis proteins, such as CheY1, and CheY2.

Initial studies in animal models demonstrated the feasibility of immunization and led to high hopes for a human vaccine [Marchetti et al., 1995]. Later mouse model immunological approaches failed to provide a satisfactory explanation for the mechanisms of protection against this largely luminal pathogen. Experimental DNA vaccine based on *H. pylori* urease B was poorly immunogenic and not protective under the conditions evaluated, such as the intragastric, intramuscular, intrarectal, and intranasal route, with CpG oligodeoxynucleotide as an adjuvant, suggesting alternative approaches [Zavala-Spinetti et al., 2006]. Intrarectal and intranasal administrations seem to be more immunogenic than other routes [Hatzifoti et al., 2006; Zavala-Spinetti et al., 2006].

Many efforts have been focused to effective vaccination by induction of a humoral and Th2 cell immune response. Mucosal immunization

with a variety of antigens in combination with mucosal adjuvants such as cholera toxin (AB5 toxin, CT), the heat labile enterotoxin (LT) of *Escherichia coli*, or Freund adjuvants, which induce a Th2 response, prevents or cures a *Helicobacter* infection, while Th1 response-inducing adjuvants enhance inflammation rather than eliminating this. A first demonstration of effective mucosal immunization eliciting a Th2 response [Ferrero et al., 1995] was based on specific salivary secretory immunoglobulin A (IgA) and serum IgG1 antibodies production after oral immunization of mice. A later study indicated that mucosal immunization with urease stimulates Th2 CD4+ T-cell response in BALB/c mice with *H. felis* infection [Saldinger et al., 1998].

Recently, transcriptome studies of gastric epithelial cell lines cultured with *H. pylori* have identified new factors involved in the inflammatory response to the bacterium [Resnick et al., 2006]. It is now proposed that non-classical immune mediators may be the key to vaccine-induced protection. In human trials of *H. pylori* vaccines some formulations are clearly immunogenic but their effectiveness remains not sufficiently tested [Kreiss et al., 1996; Michetti et al., 1999].

H. pylori AND THERAPY: OPPORTUNITIES, CHALLENGES, SHORTCOMINGS

Many rescue options have been evaluated to overcome therapeutic and preventive failures, such as the NE-2001, a novel synthetic agent [Dai et al., 2005], and the rifabutin (Mycobutin) used with success (150 mg daily) in combination with amoxicillin (1 or 1.5 g three times daily), and pantoprazole (Protonix) (80 mg three times daily) for 12 days in patients who had failed one or more eradication attempts with omeprazole, clarithromycin and amoxicillin [Borody et al., 2006]. The probiotics, for example, *Lactobacillus spp* and *Bifidobacterium spp*, may prevent infection with pathogenic bacteria both through activation the immune system of the host and through direct competition of the probiotic bacteria with the pathogen, and could present a low-cost aid in clinical practice. It was recently shown that triple therapy (amoxicillin, clarithromycin, and a proton pump inhibitor) adding bovine lactoferrin and probiotics, could improve the eradication rate and reduce side effects [De Bortoli et al., 2007].

Alternative therapeutic options to prevent or decrease *H. pylori* colonization could be antimicrobial peptides, such as magainins, bacteriocins, apidaecins, and defensins, the use of prophyrins, which exhibit antimicrobial activity through the catalysis of peroxidase and oxidase reactions and even diets based on essential oils [Gerrits et al., 2006]. Other attempts to use multi functional peptides did not obtain the desiderate effects: pyrrhocoricin, a very potent proline-rich peptide appears to kill responsive bacterial species including *E. coli*, *Salmonella typhimurium*, and *Haemophilus influenzae* but failed to bind its target in *H. pylori* [Kragol et al., 2002].

Due to difficulties in *H. pylori* treatment such as antibiotic resistance, experts proposed the development of vaccines as the best preventive measure. Some studies of vaccines failed [Zavala-Spinetti et al., 2006] but their results do not negate a potential benefit of the vaccine development; indeed, they show the need of new vaccine delivery systems and routes of immunization tested in animal models and human volunteers. It is unclear whether the failure of these studies was due to the antigens or their combination, to the oral route of administration or to the necessity of limiting the dose of the adjuvants (cholera toxin, heat LT of *E. coli*, or its mutant LTR192G) because they are too toxic. Mucosal immunization will be helpful but although *H. pylori* is a mucosal pathogen, protective immunity can be achieved by mucosal as well as parenteral administration of vaccines. Besides better antigen(s) and adjuvants, the combination with a protein in a prime-boost strategy may be needed. Also, better delivery systems need to be designed and tested for safety and immunogenicity. DNA vaccines seem to be advantageous because of simplicity in preparation and manipulation, temperature stability, and stimulation of both humoral and cell-mediated immune responses. It is important to underlie that the presence of antibodies not necessarily indicate protection, even though scientists agree that IgA, and to a lesser extent IgG, can be a marker for protective immunity. The utilization of phages (viruses that specifically infect and lyse bacteria) is another alternative solution but different problems have to be overcome, such as the allergic reactions, the appearance of mutants resistant to phages, the capture and transfer of bacterial toxins by phages. The investigation of multi-

modal therapies where vaccines are being combined with other oncological treatments such as radiation and chemotherapy is also an interesting issue.

Many powerful new approaches have shown to be especially useful in characterizing the physiology of bacteria and their interactions with their hosts. Among these, proteomic [Mini et al., 2006] and microarray analyses [Cassone et al., 2007]. This led to the new approach of "reverse vaccinology". In addition, the pan-genome approach has allowed the access to more than one genome for the same bacterial species. A structural approach using X-ray crystallography, the nuclear magnetic resonance (NMR) spectroscopy can determine the structure of antigens and may help in the rational design of target epitopes to use as vaccines candidates [Serruto and Rappuoli, 2006].

The scientific community appears to have been unduly influenced by the fact that *H. pylori* infection is widespread and often asymptomatic, as well as by the costs and complications of current treatment. In a systematic review and economic analysis from 1966 to 2004, it was showed that *H. pylori* eradication antibiotic therapy in a managed care setting of peptic ulcer disease reduces the recurrence and is cost effective [Ford et al., 2004]. So in this case the question is not whom to treat, but whom to test: in countries of low incidence of gastric cancer, persons with low socioeconomic position, blood group A, familial occurrence of gastric cancer or with premalignant lesions have priority for *H. pylori* testing.

Clinicians should still strive towards a much simple eradication strategy, but this will require investment in novel antibiotic discovery or a better understanding of the pathogenesis of the *H. pylori*. The importance of bone marrow stem cell engraftment during human gastric neoplasia is an area requiring close investigation. A recent report suggests that epithelial progenitors can function as a repository for *H. pylori* in the setting of experimental chronic atrophic gastritis [Oh et al., 2005]. Another experiment involving *H. felis* (a relative of *H. pylori*) infection of a bone marrow transplanted, irradiated, strain of mice (C57BL/6), has suggested that gastric cancer may originate from bone marrow-derived stem cells [Houghton et al., 2004]. This study does not provide definitive proof of the origin of gastric cancer. Much work is needed to show whether *H. pylori* can directly interact with these bone marrow-derived gastric stem cells and also whether these bone marrow-derived cells cause gastric cancer in humans, since no markers are available.

Complex interactions between several bacterial, host genetic and environmental factors determine whether *H. pylori* infected individuals develop gastric carcinoma. It has been demonstrated that *H. pylori*-positive persons who have concurrent helminth infection may have T helper type 1 responses and be protected against ulcer, atrophic gastritis, and gastric cancer [Fox et al., 2000]. This intriguing notion might result in a preventive approach involving the administration of low doses of immunomodulating agents to *H. pylori*-positive patients.

CONCLUSION

H. pylori might be seen as an "indicator organism" for changing microecology and disease risk, and this will also be the fate of other bacteria. In fact the Hp infection has been drastically reduced over time, but only in the developed countries. Antibiotics markets are huge and the need for new classes of antibiotics is great, but alarmingly very few new antibiotics are in the pipeline. Global regulatory agencies, such as FDA, the European Medicines Agency (EMEA), the Committee for Medicinal Products for Human Use (CHMP), Pharmaceuticals and Medical Devices Agency (PMDA) and the Japan Pharmaceutical Manufacturers Association (JPMA) with clear and constant guidelines may help the investment in antibiotics to be worth making. For example, obtaining better *H. pylori* eradication treatment efficacy in children is very important. Effective vaccines have not been developed so far, but there are so many other challenges and opportunities to target this pathogen, so why should we surrender?

ACKNOWLEDGMENTS

We regret that many relevant published works have not been cited due to space constraints. Chiara Lucchetti acknowledges the Ph.D. program in Medical Biotechnology at the University of Siena, Italy. Our study was supported by grants from Sbarro Health Research Organization and National Institutes of Health.

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