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Role of *Helicobacter pylori* infection in gastric carcinogenesis: Current knowledge and future directions

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Abstract

Helicobacter pylori (*H. pylori*) plays a role in the patho-

genesis of gastric cancer. The outcome of the infection depends on environmental factors and bacterial and host characteristics. Gastric carcinogenesis is a multistep process that is reversible in the early phase of mucosal damage, but the exact point of no return has not been identified. Therefore, two main therapeutic strategies could reduce gastric cancer incidence: (1) eradication of the already present infection; and (2) immunization (prior to or during the course of the infection). The success of a gastric cancer prevention strategy depends on timing because the prevention strategy must be introduced before the point of no return in gastric carcinogenesis. Although the exact point of no return has not been identified, infection should be eradicated before severe atrophy of the gastric mucosa develops. Eradication therapy rates remain suboptimal due to increasing *H. pylori* resistance to antibiotics and patient noncompliance. Vaccination against *H. pylori* would reduce the cost of eradication therapies and lower gastric cancer incidence. A vaccine against *H. pylori* is still a research challenge. An effective vaccine should have an adequate route of delivery, appropriate bacterial antigens and effective and safe adjuvants. Future research should focus on the development of rescue eradication therapy protocols until an efficacious vaccine against the bacterium becomes available.

Key words: *Helicobacter pylori*; Gastric cancer; Vaccine; Eradication therapy; Prevention

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Core tip: Two main therapeutic strategies could reduce the incidence of *Helicobacter pylori* (*H. pylori*)-related gastric cancer: eradication of the infection or vaccination. Success of a gastric cancer prevention strategy depends on the eradication of the infection or on vaccination before irreversible mucosal changes (severe atrophy, intestinal metaplasia or dysplasia)

have occurred. Eradication therapy results are sub-optimal due to increased antibiotic resistance in *H. pylori* and patient noncompliance. To improve the rates of eradication, rescue regimens have been developed. Concomitant and sequential protocols seem equally effective rescue strategies. An effective vaccine is not available at present, in spite of enormous effort by different researchers.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral, gram-negative, microaerophilic bacterium that plays an undisputed role in the pathogenesis of gastric and duodenal ulcers, low grade B cell gastric lymphoma (MALT lymphoma) and gastric cancer^[1]. In 1982, Warren and Marshall cultivated the bacterium. Their discovery changed the therapeutic algorithm for both peptic ulcer disease and gastric MALT lymphoma. The role of *H. pylori* in gastric carcinogenesis was clarified in 1991 when large epidemiological studies^[1,2] reported a higher incidence of gastric cancer in *H. pylori*-infected individuals, which confirmed previously published reports^[3-5]. Scientific evidence accumulated, and in 1994, *H. pylori* was named as a human carcinogen by the International Agency for Research on Cancer. The role of the infection in gastric cancer development was further supported by a study by Wang *et al*^[6] that included 2722 early gastric cancer patients and 13976 controls. This study demonstrated a higher *H. pylori* prevalence in patients with early gastric cancer than in the control group (87% vs 61%, respectively).

Gastric cancer is common; it is the third most common of all cancers among males and the fifth most common among females^[7]. The survival rate of advanced gastric cancer patients is very low (< 20%). The incidence of gastric cancer is declining in developed countries but rising in developing countries, and the overall burden of the disease is constantly increasing^[7,8].

Distinct patterns of *H. pylori* gastritis are related to different outcomes of the infection. Chronic corpus-predominant and multifocal atrophic gastritis lead to increased risk of gastric cancer formation, while antrum-predominant gastritis leads to the formation of duodenal ulcer^[9-11].

The outcome of *H. pylori* infection depends on the characteristics of the bacterium in addition to the characteristics of the host and environmental factors.

PATHOGENESIS OF GASTRIC CANCER

Gastric mucosa colonization

H. pylori infection is, in majority of cases, acquired during childhood. The bacterium has to overcome the gastric acid barrier and enter the mucus layer to complete the process of colonization^[12] and to subsequently induce damage to the gastric mucosa. Furthermore, the persistence of the infection is also important, and it reflects the ability of the bacterium to adapt to its environment and to start multiplication^[13].

To colonize the gastric mucosa the bacterium uses urease activity, motility and adhesion mechanisms^[14].

Urease activity is essential for colonization of the gastric mucosa because in the absence of urea, the bacterium can only survive in a pH range of 4.0-8.0, while in an environment containing urea, it remains viable at a low pH of 2.5. Urease catalyzes the hydrolysis of urea into ammonia and CO₂, leading to the increased pH of the bacterial microenvironment. *H. pylori* urease has a high affinity for urea, which enables the bacteria to utilize the limited amounts of urea that are present in the human stomach^[15].

H. pylori flagella-mediated motility is necessary for both colonization of the gastric mucosa and for the persistence of the infection^[14]. Expression of two flagellar proteins, FlaA and FlaB, is required for full bacterial motility^[16].

Adhesion of *H. pylori* to epithelial cells enables the bacterium to alter host cell function. Adhesion is mediated through outer membrane proteins that act as adhesins, including BabA, SabA, AlpA, AlpB and HopZ.

The interaction between the bacterium and the gastric mucus occurs through contacts between the bacterial outer membrane protein BabA^[17,18] and the Lewis^b blood group antigen. BabA is a highly variable protein that is encoded by two genes, *babA1* and *babA2*. The protein encoded by *babA2* is functionally active. A major adhesin of *H. pylori* is SabA (sialic acid binding adhesin), which interacts with sialylated structures on mucins^[14]. The proportion of sialylated structures increases in the gastric mucosa during the course of chronic *H. pylori* infection. SabA also binds to sialylated receptors on neutrophils and induces activation of the neutrophils. AlpA and AlpB are expressed in all bacterial strains and enable binding to host laminin^[14,18]. HopZ also plays role in the colonization process^[14,19].

H. pylori uses the thioredoxin system^[14,20] to induce partial breaks and changes in the polymeric structure of mucus gel. *H. pylori* infection and non-specific mechanisms of inflammation simultaneously reduce the protective capabilities of gastric mucin. One-fifth of the presenting bacteria completely adhere to the gastric surface epithelium, while the remaining bacteria reside in the surface mucus layer^[21]. The helical shape of the bacterium facilitates its penetration of gastric mucus^[22].

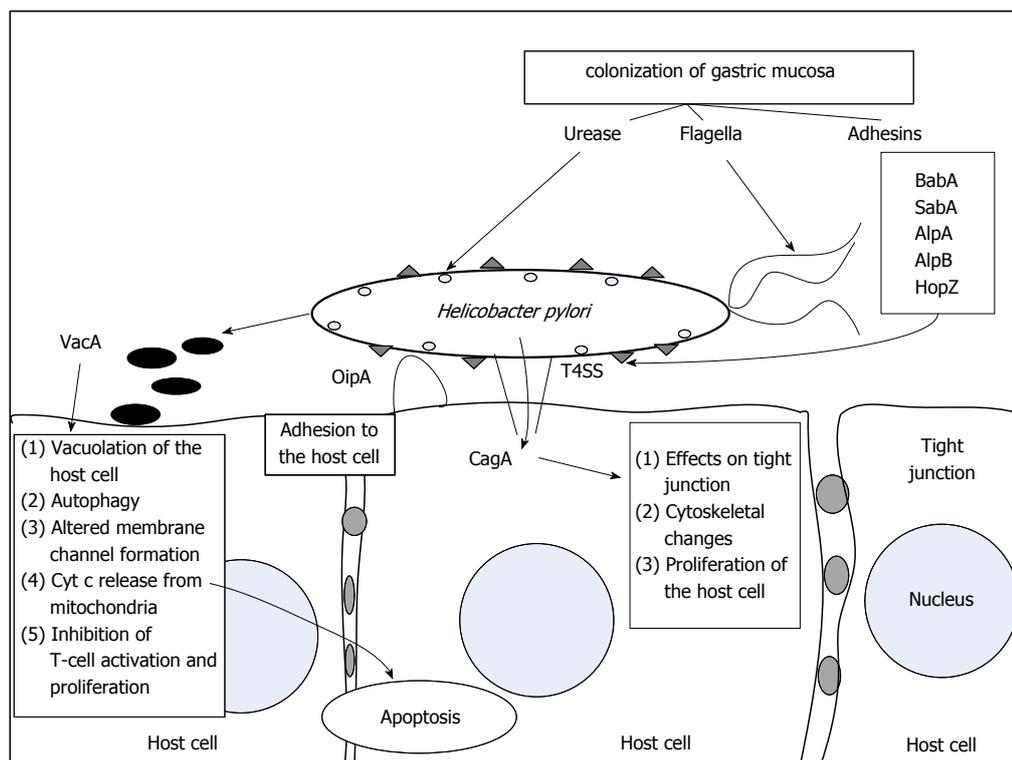


Figure 1 Mechanisms of *Helicobacter pylori* induced gastric mucosa damage.

H. pylori virulence factors

H. pylori virulence depends on the above described and other factors that are responsible for damage to the gastric mucosa (Figure 1). Epidemiological studies have identified six distinct *H. pylori* strains in different geographic regions that are related to different incidences of gastric cancer^[23]. These strains are termed hpEastAsia, hpAsia2, hpEurope, hpAfrica1, hpAfrica2 and hpNEAfrica. *H. pylori* produces various virulence factors and has the ability to modulate its reaction to the host immune response and to thereby adapt to individual host conditions^[8].

H. pylori is a highly heterogeneous bacterium^[24,25]. Virulence factors that contribute to gastric cancer development include the cytotoxin-associated gene (*cagA*) A and CagA protein (CagA), CagL, vacuolating cytotoxin (VacA) and outer inflammatory protein (OipA), while the possible role of the duodenal ulcer-promoting gene (*dupA*) remains unclear^[8].

CagA

Two distinct types of *H. pylori* are the CagA-producing (*cagA*-positive) strains and the CagA-nonproducing (*cagA*-negative) strains. In animal models, gastric cancer develops only in animals infected with *cagA*-positive *H. pylori* strains or when CagA protein is artificially introduced into the host^[26,27]. Because in humans, only some individuals infected with *cagA*-positive strains develop gastric cancer, further investigations have focused on *cagA* gene polymorphisms. The number

of repeat sequences in the 3' region of the *cagA* gene differs between *H. pylori* strains^[28]. Each repeat region of the CagA protein contains EPIYA motifs, a term used to describe a specific sequence of amino acids (Glu-Pro-Ile-Tyr-Ala). There are two EPIYA motifs in the first repeat region (EPIYA-A and EPIYA-B) and two in the second (EPIYA-C or EPIYA-D) repeat region^[24]. In Western-type *H. pylori*, CagA proteins have EPIYA ABC, ABCC or ABCCC repeat regions, while in East Asian-type *H. pylori*, CagA proteins have EPIYA ABD repeats^[8,24].

The CagA protein consists of a C-terminal region that contains the EPIYA motifs and an N-terminal region^[8]. After adhesion of the bacterium to the host cell, CagA is injected into the host cell *via* the *cag* pathogenicity island (*cagPAI*)-encoded type IV secretion system (T4SS), and electrostatic interactions with phosphatidylserine keep CagA linked to the inner leaflet of the cell membrane^[8,29]. In the cytoplasm of the host cell, CagA is phosphorylated at its EPIYA motifs^[30]. CagA alters host cell signaling in both a phosphorylation-dependent and a phosphorylation-independent manner^[8]. The induction of heme oxygenase 1 reduced CagA phosphorylation in gastric epithelial cells *in vitro*, while *in vivo* *H. pylori* diminishes heme oxygenase 1 gene expression^[8,31].

CagL

Carcinogenic and virulent *H. pylori* strains express CagL, a highly conserved protein component of T4SS that is involved in bacterial attachment to the host

cell and also in the induction of host inflammatory responses and carcinogenesis^[29]. CagL induces hypergastrinemia, which is a risk factor for the development of gastric adenocarcinoma^[8,30]. Contact between CagL and $\alpha 5\beta 1$ integrin induces IL-8 secretion from the host cell^[32].

VacA

VacA induces vacuolization and apoptosis (as a consequence of *cytochrome c* release from mitochondria) of the host cell. It is also responsible for altered membrane-channel formation and the induction of autophagy and altered host immune responses^[30,33,34], mainly through the inhibition of T cell activation and proliferation^[35]. The *vacA* gene is functional in all *H. pylori* strains and differences in its vacuolating activity have been associated with its gene structure, which varies in the signal (*s1* and *s2*), middle (*m1* and *m2*) and intermediate (*i1* and *i2*) regions^[36]. The risk of developing different gastrointestinal pathology is attributable to different combinations of *s*, *m* and *i* region subtype.

The *s1/m1* strains induce the highest level of cytotoxicity, the *s1/m2* strains induce the lowest, and the *s2/m2* strains have no cytotoxic activity (*s2/m1* strains are rare)^[37]. The risk of developing either gastric cancer or peptic ulcer disease is increased in individuals infected with *s1* or *m1* *H. pylori* strains compared with individuals infected with *s2* or *m2* strains^[8,24]. In East Asia, most *H. pylori* strains are *s1* type, and in these patients, the presence of the *m1* region is related to an increased risk for gastric cancer^[24].

The intermediate region of *vacA* is localized between the *s* and *m* regions. Type *i1* is found in all *s1/m1* strains, while all *s2/m2* strains are type *i2*. Strains that are type *s1/m2* can be either type *i1* or *i2*. Strains with the *i1* region are more pathogenic^[8]. The type of the *i*-region has a better predictive value than *s* region type in some, but not all populations^[38].

The deletion (*d*) region is localized between the *i* and the *m* regions^[39]. The *d* region can be type *d1* or *d2*. In patients infected with Western strains, the presence of the *d1* region is a risk factor for gastric mucosal atrophy. In patients infected with the East Asia type of *Helicobacter*, all strains are classified as *s1/i1/d1*^[24].

OipA

OipA is a protein that was identified in 2000^[24] and is involved in *H. pylori* adhesion to the host cell, induction of the host pro-inflammatory response and the subsequent increase in mucosal interleukin-8 (IL8) levels^[40]. Results from animal studies suggest a role for OipA in gastric carcinogenesis^[8,26].

CagA, VacA and OipA synthesis is linked. Therefore, almost all *H. pylori* strains produce either all or none of these proteins. East Asian *H. pylori* strains are highly

pathogenic and CagA, VacA and OipA-producing. According to the available data, the presence of these three genes is related to gastric cancer and peptic ulcer disease pathogenesis^[24].

DupA

Duodenal ulcer-promoting gene (*dupA*) plays a role in T4SS formation and is localized in the plasticity zone. Lu *et al*^[41] proposed *dupA* as an *H. pylori* virulence factor that is involved in duodenal ulcer pathogenesis and that confers a protective effect against gastric carcinogenesis^[8], but other studies have failed to demonstrate a relationship between this gene and any distinct gastroduodenal pathology, and they have therefore not supported this hypothesis^[23,42,43]. These results could be explained by polymorphism of the *dupA* gene^[30].

H. PYLORI, GASTRIC CANCER AND GEOGRAPHY-ROLE OF BACTERIAL STRAIN

Multilocus sequence typing of housekeeping genes revealed that there are six distinct *H. pylori* strains (hpEurope, hpEastAsia, hpAsia2, hpAfrica1, hpAfrica2 and hpNEAfrica). These strains are associated with different geographic regions and with the incidence of gastric cancer.

It is probable that housekeeping gene differences are merely markers for virulence factors that affect disease outcome^[8,23]. Initially, four main clusters were identified by Falush *et al*^[44]. HpEurope isolates were found in Europe and in countries colonized by Europeans, while the majority of isolates from East Asia were the hpEastAsia strain. HpAfrica1 is widely spread, while hpAfrica2 is found exclusively in South Africa. Two clusters were later identified: hpAsia (South and Southeast Asia) and hpNEAfrica (the predominant isolate in Northeast Africa)^[8,25].

The geographical distribution of HpEastAsia isolates is concordant with the high incidence of gastric cancer in these areas. On the other hand, in low gastric cancer incidence areas, such as Africa and South Asia, most strains are hpNEAfrica, hpAfrica1, hpAfrica2 or hpAsia2. This is a plausible explanation for both African and Asian enigma. The high incidence of *H. pylori* infection is related to the high incidence of gastric cancer in East Asia, while a low incidence of gastric cancer is observed in populations with a high prevalence of *H. pylori* infection in Africa (the African enigma) and South Asia (the Asian enigma)^[8,23].

Changes in the infected mucosa

After infection with *H. pylori*, inflammation and mucosal damage occur in the non-acid secreting gastric antrum. Over time, mucosal damage progresses into the gastric corpus. The atrophic border can be recognized

endoscopically, and the damage progresses more rapidly along the lesser curve than the greater curve, as previously reported^[44,45]. Chronic inflammation that is related to *H. pylori* affects cell differentiation and promotes metaplasia^[46-48]. As the damage spreads into the corpus, pyloric metaplasia is observed near the atrophic border. Pyloric metaplasia exhibits similar immunohistochemical characteristics to spasmolytic polypeptide/trefoil factor family 2-expressing metaplasia (SPEM), which has been described in animal models of gastric carcinogenesis^[49,50] and is probably an important step in gastric cancer formation.

DEVELOPMENT OF GASTRIC CANCER

Gastric adenocarcinoma can originate from both proximal (cardia) and distal (non-cardia) parts of stomach. Proximal and distal gastric cancers have different epidemiological and clinical characteristics^[8]. Risk factors for proximal gastric cancer include increased body weight, gastro-esophageal reflux disease and Barrett's esophagus^[8,51], while distal gastric cancer risk is increased by the presence of *H. pylori* infection^[9-11], family history of gastric cancer, low socioeconomic status, smoking and a diet rich in salty and smoked food with low consumption of fruits and vegetables^[52].

According to the Lauren classification, gastric cancer is divided into intestinal and diffuse histological subtypes. The presence of *H. pylori* infection and corpus-predominant gastritis with intestinal metaplasia leads to intestinal-type gastric cancer, whereas diffuse gastric cancer arises from non-atrophic pangastritis^[9-11].

Long-lasting precancerous processes result in intestinal-type gastric adenocarcinoma. In 1938, pathologists proposed that the presence of gastric intestinal metaplasia is related to gastric cancer^[8]. This model, now known as the Correa cascade, was reintroduced and proposed by Correa *et al.*^[53] in 1975. The authors updated their model in 1988 and 1992. In the Correa cascade, consecutive histological changes in the gastric mucosa occur, leading to gastric cancer through the following steps: normal gastric mucosa, superficial (non-atrophic) gastritis, multifocal atrophic gastritis, complete (small intestine type) intestinal metaplasia followed by intestinal metaplasia of the incomplete (colonic) type, low-grade dysplasia, high-grade dysplasia and invasive adenocarcinoma^[9-11,53]. It is now believed that intestinal metaplasia arises from SPEM and that SPEM may also provide the cells of origin for gastric cancer^[50,54]. Intestinal metaplasia is considered by some authors as a surrogate marker for the presence and extent of gastric mucosal atrophy^[55-57]. The concept of multifocal atrophic gastritis represents areas of intestinal metaplasia in SPEM-type atrophy damage^[58,59]. Nevertheless, the critically important point of no return, up to which gastric cancer prevention is possible and histological changes are

reversible, remains unidentified^[8].

HOST FACTORS

Apart from the bacterial strain, the characteristics of the host play a role in gastric carcinogenesis. Different host gene polymorphisms have been described, mainly as single nucleotide polymorphisms. These polymorphisms influence host inflammatory immune responses and affect host cell proliferation and mucosal protection. They also exert an effect on the metabolism of carcinogens and antioxidants^[8].

STRATEGIES FOR GASTRIC CANCER

PREVENTION

There are two main therapeutic strategies that could reduce the incidence of gastric cancer: eradication of an ongoing infection or immunization prior to or during the course of the infection (Figure 2). The success of a gastric cancer prevention strategy depends on its timing because prevention strategies should be introduced before the point of no return in gastric carcinogenesis. Although the exact point of no return has not been identified, infection should be eradicated before severe atrophy of the gastric mucosa develops.

ERADICATION OF *H. PYLORI* INFECTION IN GASTRIC CANCER PREVENTION-CURRENT EVIDENCE

Effects of eradication therapy for *H. pylori* infection on either invasive gastric cancer or premalignant histological lesions of the gastric mucosa have been reported in five randomized control trials (RCT)^[60-65].

Gastric cancer incidence was evaluated in a RCT conducted by Wong *et al.*^[60] that lasted for 7.5 years. Healthy individuals were randomized to receive either eradication therapy or placebo in a region in China with a high gastric cancer incidence. The study results did not demonstrate a decrease in overall gastric cancer incidence, but the results did suggest a protective role for *H. pylori* eradication in subjects without precancerous lesions^[8].

The low number of gastric cancer cases^[60,63] and the study design (it did not aim to assess gastric cancer incidence as a primary outcome) of other previously published studies^[61,62] has led to a lack of scientific evidence for the possible effects of *H. pylori* eradication on cancer occurrence. Evidence in favor of a protective effect of *H. pylori* eradication on gastric cancer formation came from a study by Ma *et al.*^[64]. They published the results of a RCT after 15 years of follow-up with 2258 *H. pylori* seropositive adults. In this study, participants were selected from the general population and randomly assigned to one of three intervention groups (*H. pylori* eradication, garlic

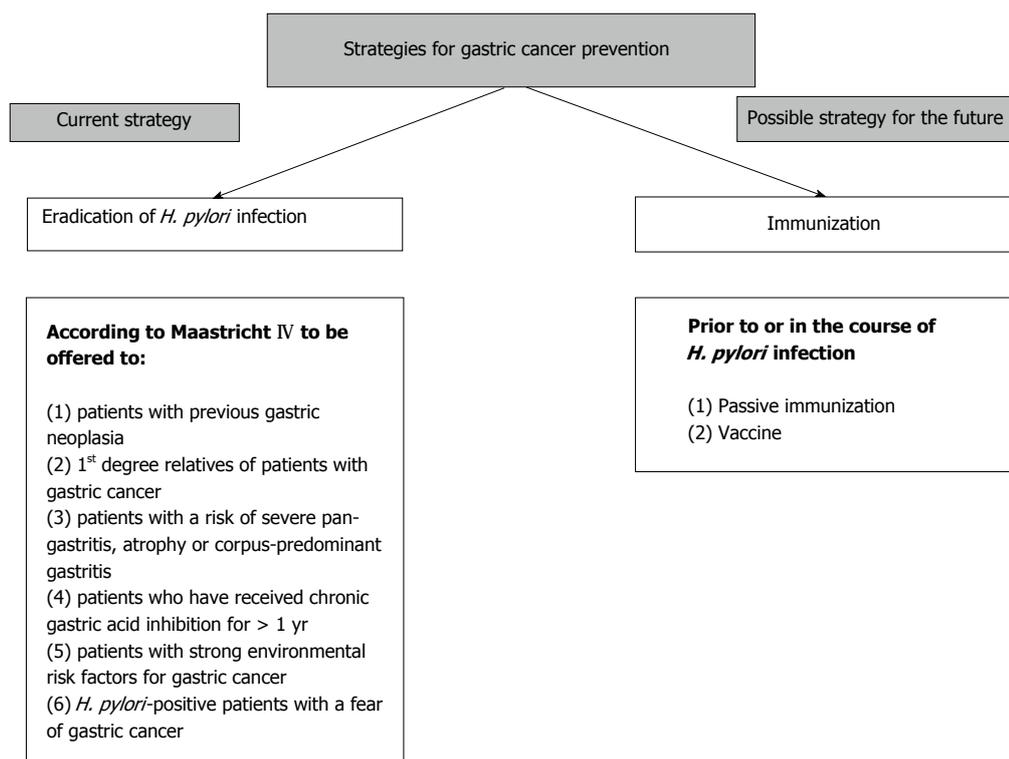


Figure 2 Strategies for gastric cancer prevention. *H. pylori*: *Helicobacter pylori*.

supplements, or supplemental vitamins) or a control group. This study demonstrated reduced gastric cancer incidence in the group of patients treated with eradication therapy for *H. pylori*.

The benefits of mass eradication in populations with high incidence of *H. pylori* was assessed by Lee *et al*^[66]. Individuals with positive *H. pylori* urea breath test underwent endoscopic screening and received eradication therapy. The success rate of the eradication therapy was 78.7%, and it led to a decrease in gastric atrophy incidence. However, no significant change in intestinal metaplasia was observed. The incidence of gastric cancer decreased by 25% during the study.

An important study from Uemura *et al*^[67] assessed the effect of *H. pylori* eradication on metachronous gastric cancer development in patients who had a previous endoscopic resection of an early gastric carcinoma. *H. pylori*-positive patients who underwent endoscopic resection were randomized to receive either *H. pylori*-treatment or no treatment. After four years of follow up, metachronous cancer was not diagnosed in any of the *H. pylori*-treated patients compared to 9% in the group that received no treatment. These findings were confirmed in a larger study by Fukase *et al*^[65], who demonstrated that eradication therapy in patients with previous endoscopic resection of early gastric cancer reduced the risk of metachronous gastric carcinoma by 65%. Current Japanese guidelines reflect an acceptance of the results of this study and suggest *H. pylori* eradication therapy after endoscopic resection of early gastric cancer^[68].

Recently published data are available from a retrospective study performed in South Korea^[69] that analyzed the relationship between the risk of metachronous gastric cancer in patients who underwent endoscopic resection of early gastric cancer and *H. pylori* eradication therapy. This study confirmed the results of Fukase *et al*^[65] and demonstrated that successful *H. pylori* eradication may reduce the occurrence of metachronous gastric cancer.

Recent trial reports^[64-66,69] have also provided evidence of a protective effect of *H. pylori* eradication in gastric cancer.

INDICATIONS FOR ERADICATION IN GASTRIC CANCER PREVENTION

According to the Maastricht IV consensus, eradication of *H. pylori* reduces the risk of gastric cancer development. Patients should be treated during the initial phase of the infection, before preneoplastic changes in the gastric mucosa occur. Authors of the Maastricht IV consensus identified individuals with an increased risk for gastric cancer. They suggest that to decrease gastric cancer risk, eradication therapy should be offered to first-degree relatives of family members with a diagnosis of gastric cancer, patients with previous gastric neoplasia who have already been treated by endoscopic or subtotal gastric resection, patients with a risk of severe pan-gastritis, corpus-predominant gastritis or severe atrophy, patients who have received chronic gastric acid inhibition for more

than 1 year, patients with strong environmental risk factors for gastric cancer (heavy smoking or high exposure to dust, coal, quartz, cement and/or work in quarries), and *H. pylori*-positive patients with a fear of gastric cancer^[70]. Two other documents, the American College of Gastroenterology guidelines^[71] and the Asia Pacific Consensus document^[72], have also recommend *H. pylori* eradication in patients with endoscopic resection of early gastric cancer.

Population screening and eradication of *H. pylori* was a matter of scientific debate until recently. Currently, the Maastricht IV consensus encourages^[70], while the Asia Pacific consensus strongly recommends, population screening and treatment for *H. pylori* in high-risk regions as a chemo-prophylactic measure for gastric cancer^[71]. The rationale behind this is the cost effectiveness of eradication therapy when compared to the cost of treatment of advanced gastric cancer.

TIMELY ERADICATION IS ESSENTIAL FOR GASTRIC CANCER PREVENTION

H. pylori eradication therapy should stop the progression of mucosal damage and reduce gastric cancer risk^[73]. Eradication of the infection stops the inflammatory process and promotes healing of gastritis and a resolution of inflammation. *H. pylori* leads to gastric cancer through the Correa cascade; therefore, when severe atrophic damage and intestinal metaplasia occur, eradication cannot reverse mucosal changes.

After eradication therapy, individuals with non-atrophic gastritis have a negligible risk of developing gastric cancer, while individuals with atrophic gastritis have an increased risk. This risk is overall lower in eradicated patients when compared to untreated patients with the same pattern of gastritis. In untreated patients, the risk for gastric cancer increases yearly as the atrophy progresses^[73], as demonstrated by Ohata *et al.*^[74] In a large, longitudinal cohort study on 4655 healthy asymptomatic subjects followed for 7.7 years, the authors aimed to determine the association between *H. pylori* infection and the progression of chronic atrophic gastritis (CAG) with gastric cancer. The authors identified 45 gastric cancer cases, none of which were *H. pylori* negative and CAG negative, during the study period. Development of CAG increased the risk of gastric cancer. Recently published data^[75] from the same group confirmed that in subjects with a serologically diagnosed healthy stomach (*H. pylori*-negative/pepsinogen within normal range and therefore CAG-negative), the cancer incidence rate was low (16/100000 person-years). On the other hand, in the individuals with an *H. pylori* infection, they observed progression of chronic gastritis and increased gastric cancer risk. In patients with no atrophy and active inflammation, gastric cancer risk was estimated at 250/100000 person-years, which is

comparable to the risk in subjects with CAG. Patients with active inflammation were at risk of diffuse gastric cancer. These results revealed that gastric cancer develops in some patients as a result of the Correa cascade, while in others it can result from a direct carcinogenic pathway based on active inflammation.

Eradication therapy, up to some point, prevents gastric cancer. Data on the possible reversion of atrophic changes in the gastric mucosa is conflicting. A longitudinal cohort study conducted by Yanaoka *et al.*^[76] with a mean follow up of 9.3 years demonstrated a significant reduction in cancer incidence after eradication in *H. pylori* positive patients with mild atrophic gastritis. The incidence in patients with persistent infection was 111/100000 person-years compared to 69/100000 among patients in whom the infection was eradicated. As expected, cancer incidence rates did not vary significantly (237 vs 223) among the patients with severe atrophy. The authors concluded that cancer development after eradication depends on the presence of extensive CAG before eradication and that *H. pylori* eradication is beneficial in subjects with mild CAG. A study by Sakakibara^[77], who followed a small group of 8 patients who were surgically treated for gastric cancer for 9 years, suggested a prompt improvement in the atrophy score (reversion of atrophic changes) in the remaining gastric mucosa following eradication therapy. The authors concluded that *H. pylori* eradication improved possible precancerous lesions in the gastric remnant.

Watairi *et al.*^[78], in a prospective study, followed 96 patients for 4 years who exhibited chronic gastritis with or without intestinal metaplasia or gastric intestinal metaplasia with dysplasia and failed to demonstrate a change in intestinal metaplasia score. Nevertheless, the authors reported a change in the intestinal metaplasia phenotype: they followed the expression of several biomarkers related to carcinogenesis and demonstrated regression in TC22-4. TC22 is a neoplastic marker that is expressed exclusively by transformed epithelial cells. Based on this finding, they suggested that the change of phenotype may be an important factor in the reduction of cancer incidence after eradication of *H. pylori*. *H. pylori* eradication prior to development of intestinal metaplasia was beneficial for patients with corpus gastritis. However, eradication in high risk patients (*i.e.*, patients with atrophy with intestinal metaplasia, especially of the incomplete type or with a history of endoscopic treatment for gastric cancer) was not beneficial^[79]. Identification of the point of no return for the development of malignancy is an important, but still unanswered, scientific goal.

SUCCESS OF ERADICATION THERAPY

There are two main underlying causes of suboptimal results of eradication therapy: *H. pylori* resistance and patient noncompliance (Figure 3).

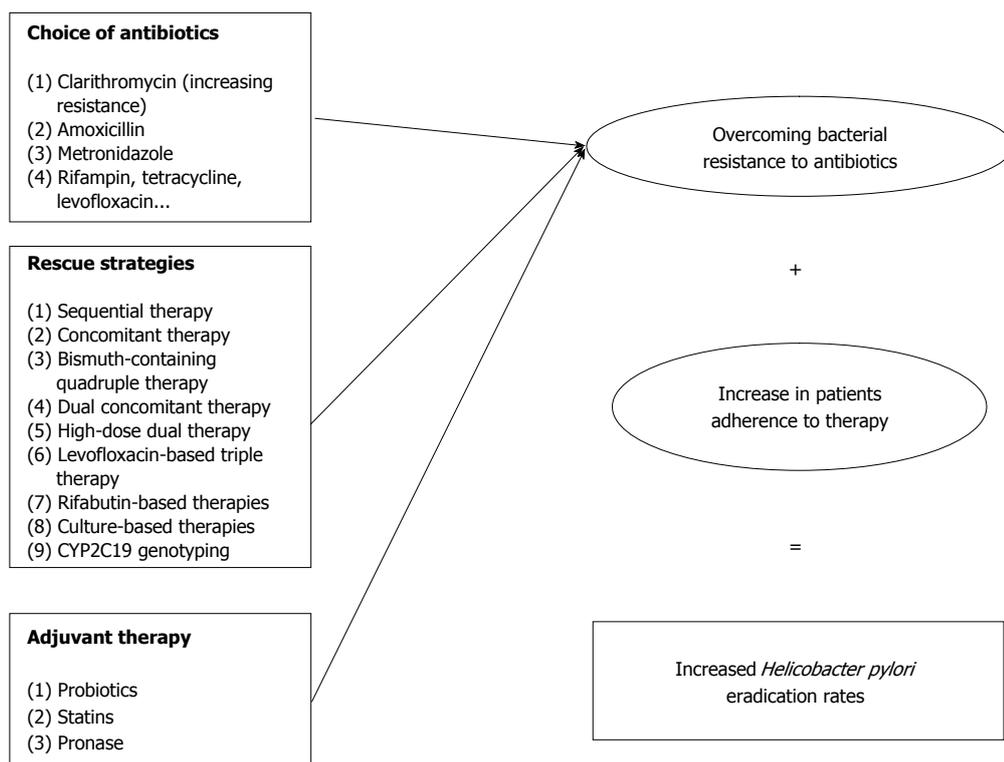


Figure 3 Factors influencing *Helicobacter pylori* eradication rates.

CHOICE OF ANTIBIOTICS IN THE LIGHT OF INCREASING BACTERIAL RESISTANCE

In the first years after the discovery of *H. pylori* and its role in the pathogenesis of major gastric diseases, eradication therapy seemed to be a safe and efficient strategy that would resolve and eradicate peptic ulcer disease, MALT lymphoma and majority of gastric cancer cases.

In 1993, an eradication protocol consisting of two antibiotics (clarithromycin and amoxicillin or metronidazole) and a proton pump inhibitor was proposed^[80,81] and confirmed as efficacious in large studies^[82,83].

Eradication therapy is effective when the mucosal concentration of the antibiotic is above the minimal bactericidal concentration (MBC) at the site of the infection for a sufficient time, which enables the eradication of all present bacteria^[84]. Macrolides (clarithromycin), beta-lactams (amoxicillin), tetracycline, metronidazole, rifampin (rifabutin) and fluoroquinolones (levofloxacin) were identified as antibiotics with the best potential for eradication of the infection. For these drugs, therapeutic doses result in a mucosal concentration above the MBC.

Selected antibiotics need to be absorbed and released in the gastric mucosa over long periods of time. The *in vitro* efficacy of aminoglycosides and bismuth salts is compromised *in vivo* by a poor rate of

absorption. In addition, some drugs from the above mentioned groups have displayed disappointing clinical effects, *i.e.*, doxycycline (tetracycline) and ciprofloxacin (fluoroquinolone). There are also other factors that are described in detail in a review by Megraud^[85] that should be considered when assessing antibiotic efficacy. These factors include the acidity of the stomach and bacterial resistance. The majority of antibiotics are not active at low pH and are only active in dividing bacteria, making the use of proton pump inhibitor (PPI) mandatory.

Clarithromycin is the basis for *H. pylori* treatment because it has MBC, good mucosal diffusion and is not affected by gastric acidity. The addition of a second antibiotic provides a high and permanent eradication rate. The second antibiotic of choice is either amoxicillin or metronidazole. Triple therapy was a standard treatment until recent years, in which we are facing increased *H. pylori* resistance to clarithromycin that is attributable to the selection of bacteria with point mutations that have occurred during replication^[86,87].

The global clarithromycin resistance rate in Europe increased from 9% in 1998^[88] to 17.6% in 2008^[89]. It has been suggested that clarithromycin resistance is the major cause of eradication treatment failure. Data on clarithromycin resistance was obtained in a study that included 18 European countries. Primary *H. pylori* antibiotic resistance rates were, for adults, 17.5% for clarithromycin, 14.1% for levofloxacin and 34.9% for metronidazole. Higher resistance rates for clarithromycin and levofloxacin are observed in Western/Central and Southern Europe (> 20%) than

in Northern European countries (< 10%). There are also data from individual countries showing a wide range of resistance rates to clarithromycin. Resistance rates range from 0% in India to 49.2% in Spain^[90,91]. There is also cross-resistance for all of the other macrolides^[85].

Resistance to rifampin, amoxicillin and tetracycline is rare^[85]. Resistance to rifampin is observed in patients previously treated for tuberculosis, while resistance to tetracycline has been reported in some^[92,93], but not all studies from Korea and Brazil^[94,95]. Resistance to metronidazole is not frequently observed and depends on the other drugs used and the length of treatment^[85].

The first Maastricht conference proposed a triple treatment including PPI-clarithromycin and amoxicillin or metronidazole^[96] that currently has an eradication rate of 70% (the aimed-for eradication rate for any protocol should be over 80%)^[97]. Possible explanations for this decrease in efficacy of the standard triple therapy include low compliance, high gastric acidity, high bacterial load, the type of strain and an increase in *H. pylori* resistance to clarithromycin. Maastricht IV therefore suggests that PPI-clarithromycin-containing triple therapy without prior susceptibility testing should not be prescribed in regions with a clarithromycin resistance rate of more than 15%-20%^[70].

RESCUE STRATEGIES FOR OVERCOMING BACTERIAL RESISTANCE

Because no new drug has been developed for this indication, a number of studies have focused on the use of different combinations of known antibiotics.

Possible scenarios proposed to overcome the problem of low eradication rates include the administration of sequential or concomitant therapy. Sequential therapy (ST) consists of a PPI and amoxicillin administered for the first five days followed by a PPI and 2 other antibiotics for the following 5 d. This sequential administration weakens the bacterial cell wall in the initial phase and helps to increase eradication rates, even in clarithromycin-resistant strains. Concomitant therapy (CT) regimen consists of all of the medication administered in ST, but given simultaneously. The efficacy of both ST^[97,98] and CT^[99,100] therapies has been supported in different studies. A recent meta-analysis provided data on the efficacy of concomitant vs sequential therapies^[101]. The analysis was based on 7 RCTs that included 2412 individuals. The sequential regimen was successful in 83.8% of patients, while concomitant therapy eradicated the infection in 86.1% of patients. The adverse events and adherence to medications were not different between the two regimens.

The idea of a bismuth-containing quadruple therapy was revisited and improved through the single pill concept. Namely, a formulation containing bismuth

salts, tetracycline and metronidazole in the same pill was developed^[102-104].

Hsu *et al.*^[105] proposed hybrid (dual concomitant) therapy consisting of dual therapy (PPI and amoxicillin for 7 d) followed by a concomitant quadruple therapy (PPI, amoxicillin, clarithromycin and metronidazole for another 7 d). The eradication rate for this treatment was over 97%.

High-dose dual therapy consists of administration of PPI and amoxicillin three times a day for 2 wk and was initially designed for areas with high resistance to clarithromycin. It provides eradication in 78.4% of patients^[106,107] and does so with fewer side effects and better compliance. The authors therefore suggested that larger studies are needed^[108].

Levofloxacin-based triple therapy consists of PPI, levofloxacin, and amoxicillin for 10 d, and the eradication rate of levofloxacin-based triple therapy ranges from 74% to 96%^[109-111]. This regimen is not recommended as the first line treatment because augmented use of quinolones for respiratory and urogenital infections increased *H. pylori* resistance to these drugs^[109]. The resistance to levofloxacin is a consequence of a point mutation in a special region, the so-called quinolone resistance determining region. There is also cross-resistance in all fluoroquinolones^[91]. Levofloxacin-based therapy is considered to be an efficient alternative regimen in populations with 15%-20% clarithromycin resistance and quinolone resistance less than 10% and is a second line treatment, according to the Maastricht IV consensus^[70]. Selection of quinolone therapy should be based on the results of antibiotics susceptibility tests or geographic resistance patterns due to the rapid increase in the number of resistant strains^[91].

Rifabutin-based therapies were introduced as rescue therapies based on the results of *in vitro* studies^[112]. A triple regimen includes amoxicillin, PPI and rifabutin, but the optimal duration of treatment is not defined and ranges from 7 to 14 d^[113-115]. Myelotoxicity is a rare but significant complication that limits its widespread use^[113]. The potential for mycobacterial resistance also limits the use of this regimen, leaving it as valid option only as a rescue treatment.

Culture-based therapies are recommended after the failure of second-line treatments. An antimicrobial susceptibility test is recommended^[69], and treatments adjusted to the results achieve more than 90% eradication rate after second-line therapy failure^[116]. The test is invasive, expensive and has low sensitivity (less than 60%)^[117]. Mixed infections with susceptible and resistant *H. pylori* strains also limit the efficacy of this therapeutic approach^[118].

The relevance of CYP2C19 genotyping as a rescue strategy for improvement of eradication rates is based on the fact that the CYP2C19 polymorphism affects the *H. pylori* eradication rate, especially by omeprazole treatment^[119]. This strategy is probably plausible in

Table 1 Adherence to eradication therapy protocols in different countries

Ref.	Country	Adherence to treatment
Wu <i>et al</i> ^[130]	Taiwan	98.2% concomitant therapy 95.7% sequential therapy
Greenberg <i>et al</i> ^[131]	Latin America (Chile, Colombia, Costa Rica, Honduras, Nicaragua, and Mexico)	92.2% overall 93.8% concomitant therapy 93% sequential therapy 89.9% standard therapy
McNicholl <i>et al</i> ^[132]	Spain	83% concomitant therapy 82% sequential therapy
Lim <i>et al</i> ^[133]	South Korea	96.2% concomitant therapy 95.3% sequential therapy
Huang <i>et al</i> ^[134]	Taiwan	94% concomitant therapy 95.3% sequential therapy
Hsu <i>et al</i> ^[135]	Taiwan	100% concomitant therapy 98% sequential therapy 99% standard therapy

Asia, where poor metabolizers account for more than 15% of all patients^[72,120].

ADJUVANT THERAPY

Adjuvant therapy in *H. pylori* eradication is likely to be of interest to researchers aiming to increase eradication rates, but the majority of data dwells on the role of probiotics. There is, however, some data suggesting a role for statins and pronase.

PROBIOTICS

Probiotics are considered safe. Thus, they are proposed as an adjuvant therapy to increase eradication rates and to decrease the side effects of therapeutic regimens, especially antibiotic-associated diarrhea. A meta-analysis published by Zhang *et al*^[121] analyzed data from 45 RCTs and revealed that the addition of probiotics to a standard therapy was associated with an increased eradication rate (82.31% in probiotic group vs 72.08% in control group) and a lower incidence of adverse events. The mechanism of this action is probably related to the ability of probiotics to induce anti-inflammatory and anti-oxidative mechanisms that regulate the intestinal microbiota. Today, urease is considered a single possible target for probiotic action^[122,123]. According to Ruggiero, it is more likely that probiotics exert indirect and non-specific, rather than direct and specific, anti-*H. pylori* activity^[124].

STATINS

HMG-CoA reductase inhibitors have many pleiotropic effects. Therefore, Nseir *et al*^[125] tested, in a small RCT, the hypothesis that the addition of simvastatin could improve eradication rates. According to this study, a better eradication rate (91% vs 72%) was observed in the group whose treatment included statin. Further

studies are needed in this field.

PRONASE

Pronase is proteolytic enzyme that causes the degradation of gastric mucus, and its addition to standard eradication therapy was investigated in 1995 and 2002^[126,127]. A single RCT by Gotoh used pronase with an eradication therapy (lansoprazole once daily, 500 mg of amoxicillin, 250 mg of metronidazole and 18000 tyrosine units of pronase thrice daily for 2 wk) and demonstrated an increased eradication rate in the group treated with pronase (ITT: 94% vs 76.5%, $P = 0.0041$)^[126]. More validation is needed because the therapeutic regimens used in these studies are not currently standard eradication therapy protocols.

ADHERENCE TO TREATMENT

Patient adherence to treatment regimens is important for the successful eradication of *H. pylori*. According to the available data, successful eradication was observed in 96% of patients who took more than 60% of the prescribed medication^[128]. Adherence to sequential therapy varies from 81%-98%, while adherence to concomitant therapy has been reported as 78.7%-100% in different studies^[129-135]. The lowest adherence is observed in Spain^[131], and the highest is observed in Taiwan^[135], as seen in Table 1.

PASSIVE IMMUNIZATION

Passive immunization is effective in the prevention and treatment of various infectious diseases^[136-138], making it a plausible strategy for the treatment of *H. pylori* as well. Data from animal studies has supported this concept, together with a previously reported protective effect from breastfeeding^[139-141]. It is probable that specific antibodies inhibit the adherence of *H. pylori*. Clinical studies have reported conflicting data. Some studies have suggested that treatment with bovine antibodies could eradicate or decrease *H. pylori* colonization density^[142,143], while others have failed to demonstrate this effect^[144,145]. The first RCT to evaluate the efficacy and safety of specific anti-*H. pylori* polyclonal bovine IgA antibodies to reduce the intragastric bacterial load and gastritis activity in humans was performed by den Hoed^[146]; the authors concluded that the antibody-based oral immunotherapy appears to be safe but ineffective because it did not significantly reduce *H. pylori* colonization density.

VACCINE

Active immunization against *H. pylori* infection would reduce the cost and potential complications of eradication therapy and is expected to lower gastric

cancer incidence. The development of a vaccine against *H. pylori* is complicated by the fact that the bacterium is noninvasive and remains strictly luminal without crossing the epithelium. An effective vaccine therefore must induce the appropriate Th memory cells that can be recruited to the mucosal surfaces. An effective vaccine against *H. pylori* should consist of appropriate bacterial antigens, an effective and safe adjuvant, and the route of delivery should be adequate. Different protocols have been tested using different antigens, adjuvants and application routes.

ANIMAL MODELS

In studies on animal models using classical immunization protocols, *H. pylori* lysates or *H. pylori* proteins were used, and plausible candidate antigens were identified (*i.e.*, urease, catalase, VacA, CagA, NapA, HpaA, AlpA and BabA). Better protection resulted from combinations of antigens^[147,148]. Recently, new bacterial antigens have been proposed as candidates for vaccine development, including 20 kD outer membrane lipoprotein Lpp20^[149], AhpC (alkyl hydroperoxide reductase)^[150] and antioxidant proteins (*e.g.*, superoxide dismutase and catalase)^[151].

Some studies that have used attenuated *Salmonella* strains, which express *H. pylori* ureA and ureB antigens, as delivery systems have demonstrated significant protection, both with intranasal and oral administration^[152,153]. Recently designed *Salmonella* vector approaches use outer inflammatory protein A^[154] for oral therapeutic immunization in addition to CagA, VacA and UreB in the vector^[155]. This OipA-*Salmonella* based approach seems to be effective at both inducing OipA-specific antibodies and reducing *H. pylori* colonization. Overall, the *Salmonella*-based approach seems to be successful in animal models.

A polio virus-based vaccination using urease B had both prophylactic and therapeutic efficacy^[156].

A multi-epitope approach is the basis of several new vaccine candidates and is described in detail in several studies. Li *et al.*^[157] used B and T cell epitopes that were generated by software prediction aimed at inducing both humoral and cellular immune response. Epivac uses proteins consisting of predicted T cell epitopes from HpaA-, UreB- and CagA^[158]-inducing serum but unfortunately no mucosal immunity. The induction of mucosal immunity could be an effective *H. pylori* vaccination protocol. Promising results were obtained in a study using chimeric flagellin consisting of the hypervariable domain of *H. pylori* FlaA and the C- and N-terminal segments of *Escherichia coli* (*E. coli*) flagellin that was designed by Mori^[159]. The chimeric flagellin was designed to maintain *H. pylori* specificity and gain TLR5 activity.

The vaccine in animal models was administered using different routes such as intranasal, oral, intramuscular, subcutaneous, rectal and intraperitoneal.

Different adjuvants have been tested in animal models, *i.e.*, cholera toxin (CT) or heat-labile enterotoxin (LT), but the clinical use of these strong mucosal adjuvants is limited in humans because of their toxicity. A possible solution to this problem is to detoxify the adjuvant while maintaining its stimulatory effect^[160]. However, these adjuvants have not been used in human clinical trials against *H. pylori*^[161].

STUDIES IN HUMANS

In humans, majority of clinical studies have used recombinant urease as an antigen. In humans, clinical trials have tested the ability of experimental vaccines to eradicate existing *H. pylori* infection or to prevent the colonization of the gastric mucosa after introduction of the bacterium in an experimental challenge^[162].

In *H. pylori*-infected asymptomatic individuals, oral immunization was well tolerated but did not lead to a specific immune response^[163], while adding LT induced an immune response^[164] and reduced *H. pylori* colonization^[165]. Diarrhea occurred as a consequence of LT toxicity, but limiting the amount of LT was not effective, in that it resolved the side effects of LT but also reduced the immune response. Rectal administration of urease and LT induced a weak immune response^[166].

Urease-expressing *Salmonella*-based delivery vectors did not prove to be effective in humans, which is in opposition to findings in animal models^[167,168], because immune reactions were undetectable. Initial data on the use of the urease-expressing *Salmonella* vaccine strain Ty21a was disappointing because the immunologic response in *H. pylori*-negative volunteers was weak. T cell memory was observed in very few subjects, and no urease-specific antibodies were detected^[168]. However, further investigation revealed that administration of multiple doses of a *Salmonella*-Ty21a based recombinant vaccine or the use of another recombinant strain that expressed the HP0231 *H. pylori* antigen resulted in the development of an immune response specific to *H. pylori* infection^[169] and a decrease in the number of bacteria in gastric biopsies, both in vaccinated subjects and in the control group. Attenuated vaccines are well tolerated and might be the correct direction for further research.

There is evidence that efficacy can be improved *via* multivalent subunit vaccines^[170]. A multivalent subunit vaccine consisting of CagA, VacA and NapA^[171] was administered intramuscularly to *H. pylori*-negative volunteers, in whom it induced both humoral and cellular immune responses without side effects. Unfortunately, protection from *H. pylori* in a clinical setting did not differ between the placebo and the vaccine groups^[172].

Recently, CagL protein was proposed as promising candidate for use in a subunit vaccine^[173].

MUCOSAL ADJUVANT

The use of strong mucosal adjuvants that have been tested in animal models is limited in humans because of their toxicity (*i.e.*, CT and LT).

In the field of mucosal adjuvants, important results have been published in recent years. Nedrud *et al.*^[174] demonstrated that the intranasal administration of a mucosal adjuvant, CTA1-DD (a derivative of the cholera toxin), is safe, effective and protected against a live *H. pylori* challenge in mice. The use of heat shock proteins (Hsp) as the mucosal adjuvant in a *H. pylori* vaccine has also been reported^[175]. This vaccine was administered *via* respiratory route and induced systemic and mucosal antibodies; protective immunity was generated with a milder post-immunization inflammation. Nevertheless, sterilizing immunity was not achieved^[175]. Promising results were obtained when a nontoxic double mutant of an *E. coli* toxin (R192G/L211A) (dm2T) was used as the mucosal adjuvant^[176]. Altman *et al.*^[177] suggested that carbohydrate-based vaccines against *H. pylori* should use dextran-based conjugates, and Zhang *et al.*^[178] suggested recombinant *Lactococcus lactis* as a vector because the bacterium is already used in dairy products.

ROUTE OF ADMINISTRATION

A report from Shirai *et al.*^[179] indicated that salivary antibody formation is a critical factor for successful vaccination against *H. pylori*. This was supported by the findings of Ng *et al.*^[180], who confirmed increased levels of salivary IgA without an increase in mucin production or cytokine level. Intranasal application in animal models seems to be promising and effective^[174]. A multivalent subunit vaccine consisting of CagA, VacA and NapA^[172] was administered intramuscularly. The sublingual^[176] route appears to be promising, while an oral route of administration has repeatedly been tested with variable effects that depend on the antigen and the adjuvant or vector used^[163-165,169,178]. Rectal administration was tested once and the results were somewhat disappointing^[166].

OVERCOMING IMMUNITY ISSUES

To develop an effective vaccine for *H. pylori*, a better understanding of protective immune responses from data on animal models and a better understanding of host responses is needed. The identification of the underlying mechanisms that prevent a host from clearing the infection could help in the development of an effective vaccine.

The T helper cell response is an important part of the protective immune response because data on animal models suggest that mice lacking antibody molecules, including mucosal immunoglobulin IgA, are well-protected by vaccinations^[181-183], while mice with deficient cellular immunity are not protected^[182,184].

These data suggest that the T helper cell response is crucial for the induction of protective immunity, even in the absence of other forms of adaptive immunity^[161]. This is based on the fact that Th1 or Th17 cell-induced increases in inflammation lead to the development of protective immunity. Therefore, a successful vaccine should induce a strong Th1 or Th17 response. According to Zawahir, possible multiple effector mechanisms that can eradicate *H. pylori* are not adequately defined. Therefore, vaccine efficacy could be improved through the enhancement of Th1 or Th17 responses to *H. pylori*^[161]. It seems that the host immune response is age dependent, because a weaker Th1 response is observed in children^[185].

In isolated cells, the removal of CD25⁺ T cells before *H. pylori* stimulation increases the IFN γ response^[186]. The results obtained in an animal model revealed that T regulatory cells suppress the active host immune response to *H. pylori* infection^[186-190]. Because the majority of infected individuals do not have an *H. pylori*-associated disease, the host might not recognize the bacterium as dangerous and may suppress the immune response. Another possible target for improvement of vaccine efficacy is therefore overcoming the host predisposition to reducing immune responses to bacteria. A vaccine should increase the host cellular immune response, as supported by findings in animal models where bacterial load was reduced using IL-17^[191] and IL-12^[192].

Recent publications by Muhsen *et al.*^[193,194] demonstrated higher seroconversion rates to a typhoid vaccine in *H. pylori*-infected subjects, and the authors also demonstrated that gastric *H. pylori*-associated inflammation promoted seroconversion. Based on these findings, the concept that active *H. pylori* infection could be beneficial for the efficacy of other vaccines becomes attractive, and further research is needed in this area.

CONCLUSION

Timely eradication of *H. pylori* infection is, at present, the single available evidence-based strategy for reducing *H. pylori*-related gastric cancer risk, incidence, and subsequent morbidity and mortality. It is mandatory to eradicate the infection before irreversible mucosal damage occurs. Eradication should precede the development of severe atrophic changes.

Future possible strategies include the development of an effective vaccine. It is rather disappointing that although some of the experimentally tested vaccines have shown promising results, there is limited funding and financial support from the pharmaceutical industry. At the moment, another possible strategy is the development of new antimicrobial agents that would be effective for eradication therapy, but these are long-lasting and time-consuming studies. Therefore, at present, it is advisable to insist on patient compliance

during eradication therapy because this is the area where improvement is possible.

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