Chapter

Helicobacter pylori Challenge Vaccine for Humans

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Abstract

Helicobacter pylori infect during childhood and are typically present for life, despite a vigorous host immune response, which includes the invading pathogen being coated with antibodies. This bacterial longevity indicates the development, on the part of the pathogen, of a range of processes for evading effective host immunity. Since its discovery 25 years ago, significant progress has been made in understanding the virulence factors and several aspects of the pathogenesis of *H. pylori* gastric diseases. The prevalence of antimicrobial drug resistance is so high that all patients infected with *H. pylori* should be considered resistant infections. The most severe consequence of *H. pylori* infection, and the key reason a vaccine is required, is gastric cancer, globally the third leading cause of death due to cancer. Patients typically present with gastric cancer without knowing they are infected; eradication likely has little effect by this time. Vaccine against *H. pylori* that reduces the incidence of gastric cancer will probably be cost effective in developed countries. Several vaccines were successfully tested in different experimental animal models, but translation into an efficacious human vaccine has been unsuccessful.

Keywords: *Helicobacter pylori*, *H. pylori* vaccine, gastric disease, gastric cancer

1. Introduction

Helicobacter pylori (H. pylori) is a gram-negative, motile, spiral, or curved bacterium that colonizes the human gastric mucosa about 50% of the human population [1]. *H. pylori* induces the development of a peptic ulcer, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer [2]. Globally, gastric cancer ranks as the third leading cause of death from malignancy [3].

The prevalence of *H. pylori* infection in developed countries ranges from 30 to 40%, while it can reach 80% [4–8]. Hygiene conditions and socioeconomic status affect the incidence of *H. pylori* infection [5]. *H. pylori* infection is thought to result from direct human-to-human transmission through oral, fecal, or both. The infection is generally acquired during childhood and then increases gradually with age. *H. pylori* is commonly transmitted from mother to child [1] This evidence is supported by the ability to grow *H. pylori* from vomit or oral area and analysis of bacterial strains indicating general vertical transmission between mothers and their offspring [1, 9, 10]. If left untreated, most *H. pylori* infections last a lifetime.

It is challenging to eradicate *H. pylori* because of its high antimicrobial resistance. In addition, most of the infected people are asymptomatic. The costs for adequate diagnostic tests and pharmacological eradication will be enormous. Treatment of *H. pylori* requires a multidrug regimen because the organism colonizes

beneath the mucous layer, which reduces the direct effect of antibiotic penetration [11]. Resistance is also a problem with some commonly used antibiotics, namely metronidazole, amoxicillin, erythromycin, and clarithromycin [11–15]. Unfortunately, increasing antibiotic resistance is beginning to affect the efficacy of treatment, and, despite the impact of *H. pylori*, preventive vaccination strategies are still lacking. Until now, there is no recommended *H. pylori* vaccine available. Here, we provide an overview of the constraints and challenges in the manufacture of vaccines against *H. pylori*.

2. Morphology of *H. pylori*

H. pylori is a gram-negative, non-spore-forming bacterium, spiral-shaped or rods. It will turn into a coccoid form in unfavorable conditions, a form of defense resistant to conditions [16]. Several studies have shown that the coccoid form of *H. pylori* decreases morphological manifestations and remains alive and metabolizes actively, although it cannot be cultured [16, 17].

These bacteria have flagella that allow high motility and are microaerophilic. *H. pylori* has 1–6 sheathed flagella at the terminals, which are lophotrically arranged. Other Helicobacter species have flagella that are not sheathed [18]. The length of this bacterium is between 2.5 and 3.5 m, and the width is 0.5–1.0 m. *H. pylori* can grow well at 35–37°C and produces the enzyme catalase, cytochrome oxidase, urease, alkaline phosphatase, and glutamyl transpeptidase. The proper place for bacteria to live in the human body is the antrum. The number of *H. pylori* that appears to show the ability to adapt to certain areas, for example, in the human stomach on the surface of epithelial cells and the mucous layer [16, 18].

These bacteria have the same layer composition as other gram-negative bacteria: an inner membrane, periplasm with peptidoglycan, and an outer membrane. The outer membrane is composed of phospholipids and lipopolysaccharides (LPS). *H. pylori* LPS variation plays a role in population heterogeneity and allows adaptation to changes in gastric mucosal conditions. Outer membrane phospholipids contain cholesterol glucoside, which is very rare in bacteria [18].

3. *H. pylori* adaptation and colonization

After entering the stomach, *H. pylori* performs four colonizing stages, including surviving against gastric acid, moving toward gastric epithelial cells, attaching to gastric epithelial cells, and releasing toxins, causing tissue damage [19]. The enzyme urease is an essential factor for *H. pylori* colonization of the gastric mucosa. This enzyme converts urea (a product secreted by gastric cells) into ammonia and carbon dioxide. Ammonia can increase the pH of the gastric mucosa around bacterial cells. Therefore, this enzyme neutralizes the acidity of the stomach and aids in colonization. However, this enzyme can also stimulate monocytes, neutrophil chemotaxis, and stimulate cytokine production [20].

H. pylori uses flagella and specific chemoreceptors, TIpB, to move toward gastric epithelial cells near-neutral pH. The circular motion is facilitated by the helical shape of the bacteria, which can pass through the dense mucosal layer [20]. These bacteria can penetrate the mucus layer through the production of a protein called collagenase/mucinase. Collagenase/mucinase functions to liquefy mucin, reducing viscosity and allowing these bacteria to move more freely to reach epithelial cells. In addition, *H. pylori* produces alpha-carbonic anhydrase (α-CA), which helps

urease convert carbon dioxide into bicarbonate. Bicarbonate is a weak base that can neutralize stomach acidity [19].

After successfully passing gastric acid, *H. pylori* attaches to gastric epithelial cells with the help of adhesins. Adhesins bind to receptors on the gastric mucosal surface. The majority of adhesins are *H. pylori* outer membrane proteins (OMPs) [21]. These adhesives include BabA (Blood Group Antigen-Binding Adhesin), SabA (Sialic acid-binding Adhesin), AlpA/B (Adherence-associated Lipoproteins A/B), HopZ, OipA, and HpaA. Receptors for BabA, Sab A, and AlpA/B adhesins include the Lewis human blood antigen group b (Leb), sialyl Lex, and laminin. Meanwhile, the HopZ and OipA receptors have not been identified [21–23].

The LPS chemical structure of several *H. pylori* strains resembles the Lewis x and Lewis y blood antigen groups expressed in the gastric mucosa. It serves to downregulate the immune response in patients with acute and chronic infections [24]. Specific modification of the LPS molecule allows molecular mimicry and alteration of the structural components of lipid A, leading to low endotoxic activity. The *H. pylori* membrane is coated with the same molecules on the host cell as plasminogen and cholesterol that protect the bacteria from host cell recognition. The high genetic diversity of bacteria allows for rapid adaptation to environmental changes [20]. Phospholipase *H. pylori* produces products such as lysolecithin, which interfere with the protective layer of phospholipids that are abundant in the apical membrane of mucus cells [24].

H. pylori has a Cag Pathogenicity Island (CagPAI), which is associated with the development of chronic active gastritis, peptic ulcer, and atrophic gastritis with an increased risk of gastric cancer. CagA is a virulence factor located at one end of CagPAI, a 40 kb of the *H. pylori* genome. Cag PAI encodes 31 genes that make up the type IV secretory system (T4SS), which injects CagA, an oncoprotein, into the cytosol of gastric epithelial cells [23]. Upon entry into gastric epithelial cells, CagA undergoes Src-dependent tyrosine phosphorylation and activates SHP-2, which leads to dephosphorylation of host cell proteins and changes in cellular morphology. Translocation of CagA protein supports the release of essential nutrients to the apical side of epithelial cells, either by induction of inflammation or by host cell depolarization [24]. Apart from CagA, peptidoglycan is also transported into the host cell *via* T4SS and outer membrane vesicles. Peptidoglycan activates the intracellular Nod1 receptor, which activates NF-kB (a transcription factor associated with epithelial gene expression and regulates the expression of various proinflammatory cytokines). Modifications in these settings are important to dampen the host immune system and contribute to bacterial persistence [25].

The second most studied *H. pylori* virulence factor is VacA. VacA is a 140-kDa polypeptide. The gene-encoding VacA is present in all *H. pylori* strains and exhibits allelic diversity in three major regions, namely, s (signal), the i (intermediate), and m (middle). As a result, the cytotoxic activity of the toxin varies between strains. Different combinations of alleles from each region (s1, s2, i1, i2, m1, m2) that exist result in different abilities of VacA toxin to stimulate vacuolation in epithelial cells [24]. VacA can bind to several epithelial cell surface molecules, including transmembrane protein receptors-type tyrosine-protein phosphatase (PTPRZ1), fibronectin, EGFR, CD18 on T-cells, and various lipids and sphingomyelin [24]. VacA can induce vacuolation and several cellular activities, including membrane channel formation, the release of cytochrome c from mitochondria leading to apoptosis, and binding to cell membrane receptors followed by initiation of the proinflammatory response. VacA can also inhibit the activation and proliferation of T and B cells [23]. VacA can also inhibit the phagosomal maturation of macrophages and induce macrophage apoptosis [24].

4. Natural history of *H. pylori* infection

The natural history of *H. pylori* infection can be divided into two stages. The first is the acute phase, where bacteria multiply and cause gastric inflammation, hypochlorhydria, and some gastrointestinal symptoms such as fullness in the stomach, nausea, and vomiting [26]. This phase often occurs during childhood and is almost difficult to diagnose. After several weeks, the chronic phase begins with a reduced inflammatory response, and gastric pH normalizes, then becomes asymptomatic. Colonization of *H. pylori* in the gastric mucosa causes infiltration of neutrophils and mononuclear cells in the antrum and body, leading to chronic inflammation. When colonization becomes persistent, there is a close correlation between the level of acid secretion and the distribution of chronic gastritis. The most common feature is non-atrophic gastritis with normal acid secretion in asymptomatic subjects. Antral-dominant gastritis is associated with hyperchlorhydria and duodenal ulcers, whereas dominant corpus gastritis causes hypochlorhydria, gastric atrophy, intestinal metaplasia, and an increased risk of distal gastric cancer [27].

H. pylori colonization causes a degree of inflammation in the gastric mucosa. Reactive oxygen species produced from polymorphonuclear after activation by *H. pylori* induce gastric mucosal injury. Polymorphonuclear cell infiltration of the gastric mucosa leads to the development of early *H. pylori* infection lesions called active chronic gastritis, the natural history of *H. pylori* infection [16].

Acid secretion is affected by *H. pylori* infection, which is also associated with dyspepsia. Patients with functional dyspepsia and *H. pylori* infection had a fourfold increase in acid secretion. In contrast, asymptomatic *H. pylori*-positive individuals had only a 2.5-fold increase in acid secretion. Acid secretion during *H. pylori* infection depends on the spread of gastric mucosal atrophy and the local stage of inflammation, which is determined by interactions between the host, bacteria, and environmental factors. In *H. pylori*-infected patients with dominant antral gastritis without corpus atrophy, acid secretion was more elevated than in uninfected normal mucosa. This is a potential cause of dyspeptic symptoms, such as epigastric pain or burning. In contrast, when the atrophy extends to the corpus mucosa (fundic glands), reduced acid secretion is due to direct damage to parietal cells in the corpus, associated with gastric ulcers and gastric cancer [16, 28, 29].

5. Immune evasion in persistent infection with *H. pylori*

The primary defense barrier against *H. pylori* is the mucus secreted by epithelial cells and innate immune cells in the lamina propria [30]. *H. pylori* and its products can directly contact lamina propria immune cells, resulting in an influx of immune cells that include neutrophils, macrophages, dendritic cells, lymphocytes, and associated innate and adaptive immune responses [31]. Toll-like receptors (TLRs) are a major group of Pattern Recognition Receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). Bacterial lipopolysaccharide (LPS), peptidoglycan, lipoprotein, lipoteichoic acid, and unmethylated CpG-rich regions of DNA are the main targets of TLRs [31]. Studies have shown that *H. pylori* has managed to escape the introduction of TLRs. For example, TLR4 runs the well-described LPS recognition [32].

H. pylori subverts the adaptive immune response by modulating effector T cells [31]. During *H. pylori* infection, the frequency of CD4+ T cells in the gastric lamina propria with a memory phenotype increases and polarizes to a Th1/Th17 phenotype, but these T cells are hyporesponsive to this bacterium [33]. Because

this hyporesponsiveness contributes to chronic infection, attempts have been made by *H. pylori* to downregulate the T-cell response. *H. pylori* also manipulate T cell function by eliciting regulatory T cells (Tregs), frequently found in these patients [34]. Unusual Tregs activation by microbial antigens may provide a mechanism for preventing *H. pylori* from the immune response. Gamma-glutamyl transpeptidase (GGT) and VacA from the *H. pylori* molecule indirectly affect T lymphocyte activity and promote the differentiation of effector CD4+ T cells into Tregs. [35].

6. Current status and challenges of *H. pylori* vaccine candidate development

Since the early 1990s, vaccines based on various antigens, adjuvants, and routes of administration have been evaluated. The mucosal route of administration, especially the oral route, is the most suitable route for vaccination against *H. pylori* infection to induce an effective immune response at the site of infection. Until now, many vaccine candidates have been developed at the preclinical stage. In comparison, the most advanced candidate for the *H. pylori* vaccine is in phase 3 clinical trials (**Table 1**).

6.1 Oral recombinant *Helicobacter pylori* vaccine

This vaccine (UreB/LTB fusion vaccine) is a recombinant oral *H. pylori* vaccine using a urease B subunit (gene derived from *H. pylori* 9803) fused with heat-labile enterotoxin B subunit (gene derived from *E.coli* H44815) developed by Third Military Medical University and Chongqing KangWei Biotechnology in China. A randomized, double-blind, placebo-controlled phase III clinical trial was conducted in Ganyu County, Jiangsu Province, China. Vaccination was administered orally

Candidate vaccine	Country of the laboratory	Trial status	Prophylactic /therapeutic	References
Candidate vaccine: Oral Recombinant Helicobacter Pylori Vaccine	China	Phase III	Prophylactic	[36]
Imevax/IMX101	Germany	Phase I	Prophylactic	[37]
HelicoVax	USA	Preclinical	Therapeutic	[38]
Recombinant CTB-UreI-UreB (BIB)	China	Preclinical	Prophylactic	[39]
Recombinant *Vibrio cholerae* expressing *H. pylori* HpaA antigen	Sweden	Preclinical	Prophylactic	[40]
CTB-Lpp20	China	Preclinical	Prophylactic /Therapeutic	[41]
MCRI (Murdoch Children Research Institute /Gastric Cancer Vaccines)	Australia	Preclinical	Prophylactic	[42]
H. pylori Vaccine (NCT00736476)	Germany	I/II	Prophylactic	[43]

Table 1.
Summary of H. pylori *vaccine development status.*

in 3 doses on days 0, 14, and 28. In this study, oral administration of the *H. pylori* vaccine provided good protection against *H. pylori* infection in children aged 6–15 years up to 1 year after vaccination. Although the estimated point of protection for the vaccine later shows a slight decrease in efficacy, overall safety can last up to 3 years. All side effects are mild and improve within 24 hours. The most common reaction is vomiting, followed by fever and headache [36].

6.2 Imevax/IMX101

Imevax has completed a phase I clinical trial with IMX101. The vaccine consists of the *H. pylori* antigen-glutamyltranspeptidase (GGT), an outer membrane protein, and a mucosal adjuvant. The main reason for the failure of previous vaccines to provide complete protection is the immune evasion strategy possessed by *H. pylori* [35].

One of the most important is GGT, which appears to have relatively immunosuppressive solid activity. Therefore, this vaccine aims to target and neutralize these defense mechanisms, potentially enabling a more effective protective immune response against other antigenic components of the vaccine. The phase I clinical trial of Imevax IMX101 was conducted in a multi-center, randomized, double-blind, and adjuvant-controlled study conducted on adult volunteers aged 18 to 50 years to evaluate safety, tolerability, and efficacy. Volunteers consisted of negative people for *H. pylori* and healthy people infected with *H. pylori*. IMX101 vaccine is administered sublingually and intradermally [37].

6.3 HelicoVax

In the study by Steven F. Moss et al., they designed two DNA vaccines, namely HelicoVax A and HelicoVaxB, each containing a set of 25 different HLA class II epitopes. Steven F. Moss et al. used C57BL/6 mice. At six weeks, mice were infected with *H. pylori* strain SS1 in 0.1 ml PBS, 3 times in 1 week. DNA vaccines are administered intramuscularly and intranasally. The test results show that there is promising therapeutic efficacy for the development of an epitope-based mucosal vaccine against *H. pylori*.

6.4 Recombinant CTB-UreI-UreB (BIB)

Epitope vaccines are a promising option for protection against *H. pylori* infection. Research conducted by Jing Yang et al. developed a multi-epitope vaccine by linking the cholera toxin B (CTB) subunit, two antigenic fragments of *H. pylori* urease I subunit (UreI20–29, UreI98–107), and 4 *H. pylori* antigenic fragments. Urease B subunit, (UreB12–23,UreB229–251,UreB327–400,UreB515–561) produces recombinant CTB-Urel-UreB (BIB). This vaccine's protective effect against *H. pylori* infection was evaluated in BALB/c mice. Significant protection against *H. pylori* infection was achieved in BALB/c mice immunized with BIB, rIB plus rCTB, and rIB. Induction of substantial protection against *H. pylori* was mediated by specific serum IgA and mucosal sIgA antibodies and a mixed response of Th1/Th2/Th17 cells. This multi-epitope vaccine can be a promising vaccine candidate that helps control *H. pylori* infection [39].

6.5 Recombinant Vibrio cholerae expressing *H. pylori* HpaA antigen

The vaccine was designed by constructing and characterizing the faster-growing O1 *Vibrio cholerae* strain of *H. pylori* as a vector for the *H. pylori* antigen that might

be used as a vaccine strain against *H. pylori*. Joshua Tobias et al. developed the technology of over-expressing enterotoxigenic *E. coli* (ETEC) colonization factor antigens (CFs), the main virulence factor of ETEC, in heterologous bacterial strains including *V.cholerae*. Due to the extracellular nature of *H. pylori*, the bacteria colonize the epithelial surface and coat the gastric mucosal lining and areas of gastric metaplasia in the duodenum. Joshua Tobias et al. constructed a *V.cholerae* strain that overexpressed HpaA, as a surface antigen and *H. pylori*-specific lipoprotein known to mediate *H. pylori* colonization in the rat stomach and be a protective antigen against *H. pylori* infection in animal models, singly or in animal models. Concurrently with different ETEC CFs can promote bacterial binding to the small intestinal mucosa. Specific strains were developed and characterized to the level of surface expression of HpaA, and the capacity to induce an immune response against *H. pylori* in mice after oral immunization [40].

6.6 Lp220 vaccine

Epitope vaccine is a potential vaccine as a prophylactic and therapeutic vaccine against *H. pylori* infection. Lpp20 is one of the main protective antigens that trigger immune responses after *H. pylori* invades the host and is considered an excellent vaccine candidate for the control of *H. pylori* infection. This epitope vaccine consists of a mucosal adjuvant cholera toxin B subunit (CTB) and three Lpp20 epitopes that have been identified (one B cell epitope and two CD4+ T cell epitopes) for efficacy in mice. An epitope vaccine consisting of CTB, one B cell, and 2 CD4+ T cell epitopes of Lpp20 was prepared and named CTB-Lpp20, which is expressed in *Escherichia coli* and used for immunization BALB/c mice *via* intragastric. The CTB-Lpp20 epitope vaccine has good immunogenicity and immunoreactivity and can produce high specific antibodies against Lpp20 and the cytokines IFN-c and IL-17. In addition, CTB-Lpp20 significantly decreased *H. pylori* colonization in mice. This protection correlates with IgG, IgA, and sIgA antibodies and Th1-type cytokines [41].

6.7 MCRI (Murdoch children research institute/gastric cvaccines)

MCRI (Murdoch Children Research Institute) developed a new vaccine strategy that prevents *H. pylori*-induced inflammation. The vaccine candidates are recombinant HtrA, a-55 kDa protein, and the only serine protease produced by *H. pylori*. HtrA is expressed and secreted on the bacterial surface, and is essential for the survival of *H. pylori*. MCRI investigators have shown that mice vaccinated with HtrA protected against *H. pylori*-induced inflammation compared with controls. *H. pylori* HtrA destroys the epithelial barrier by cleaving E-cadherin thereby opening junctions between gastric epithelial cells. The leaky epithelium allows a number of bacteria to enter the epithelial barrier to the tissue, interact with immune system cells, and cause gastritis. MCRI investigators found that serum from mice vaccinated with HtrA neutralized HtrA protease activity *in vitro* [42].

6.8 *H. pylori* vaccine (NCT00736476)

The vaccine consists of three recombinant *H. pylori* antigens vacuolating cytotoxin A (VacA), cytotoxin-associated gene A (CagA), and neutrophil-activating protein (NAP) that prevent infection in animal models and are well tolerated and highly immunogenic in adults receiving healthy. In this phase 1/2 randomized, single-center, unsupervised, placebo-controlled study, healthy nonpregnant adults aged 18–40 years who were confirmed negative for *H. pylori* infection were

randomly assigned (3:4) to three intramuscular doses placebo or vaccine at 0, 1, and 2 months [43]. Previously, three recombinant antigen vaccines were tested relevant to *H pylori* virulence—CagA, VacA, and NAP—in phase 1 clinical study [44]. Compared with placebo, the vaccine did not provide additional protection against *H. pylori* infection after challenge with CagA-positive strains, despite an increased systemic humoral response to key *H. pylori* antigens. The vaccine induces high-titer IgG antibodies specific for CagA, VacA, and NAP and a robust antigen-specific T cell response, but this is not sufficient to eliminate *H. pylori* [43].

7. Conclusion

The best preclinical results are obtained from vaccines that often induce a T-cell-mediated immune response rather than humoral immunity. Th1 and Th17 responses in the stomach are more protective. The mechanisms of *H. pylori* persistence and the utilization of multiple mechanisms to overcome adaptive immunity are recognized as essential barriers to vaccination. Several vaccines were successfully tested in different experimental animal models, but translation into an efficacious human vaccine was unsuccessful. A better understanding of the immune response generated by natural *H. pylori* infection and the mechanism by which the bacteria survives is needed for the development of human vaccines. Future vaccines for the prevention of *H. pylori* infection should be conducted in children, where infection occurs naturally. Therefore, prophylactic vaccines may need to be given to children in the first few years of life to reach the maximum number of target groups when uninfected, but the health benefits will emerge five decades later.

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Conflict of interest

The authors declare no conflict of interest.

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