

A Recombinant Vaccine for *Helicobacter pylori*

Submitted for editing November 16, 2007

MIC 519/419H

Literature Review

Epidemiology

Ulcers were once thought to be caused by stress, spicy foods, or acidity of the stomach (Marshall and Warren, 1984). However, it has been discovered that ulcers are caused by the bacterium *Helicobacter pylori*. *H. pylori* can also cause chronic infections such as active gastritis, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma. All people are considered to be part of the risk group, but those that live in developing countries are at a higher risk. It is unknown how *H. pylori* is transmitted, but humans are the only known reservoir.

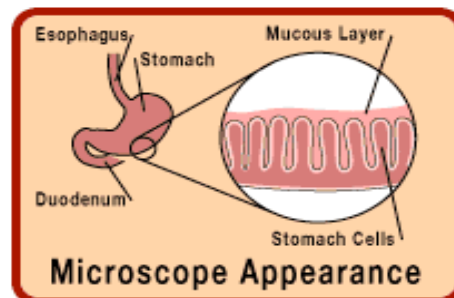
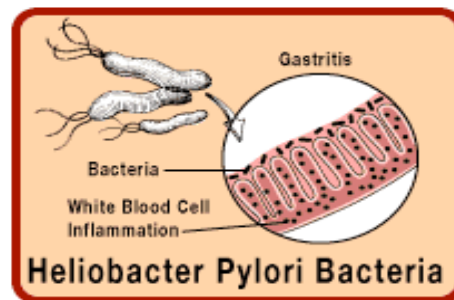
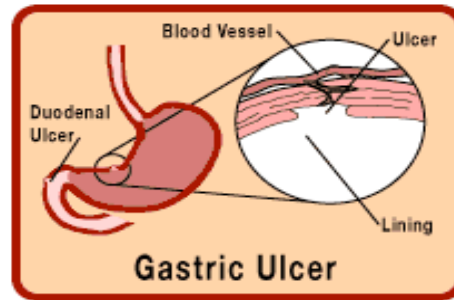
The occurrence of disease increases with age and the elderly are at the most risk of death. Death from ulcers occurs from damage to the peritoneal cavity or from bleeding without a way to stop it. The prevalence of ulcers decreases when there is an increase in hygiene standards. Recent findings have shown that in developed countries there has been a steady decline in mortality due to *H. pylori* (Sonnenberg, 2007). This is likely due to a decrease in infection from increasing standards of hygiene. However, in less developed countries these infections are still a problem.

Developing an effective vaccine to *H. pylori* is important for a number of reasons. There is increasing resistance to commonly used antibiotics such as metronidazole, clarythromycin, and amoxicillin (Koletzko *et al.*, 2006). Treatment for *Helicobacter pylori* once included antacids and a treatment that focused on preventing acid production, which consisted of bland foods and often hospitalization. Unfortunately, the recurrence of ulcers was high among patients. Ten days to two weeks worth of antibiotics, and ranitidine bismuth citrate, bismuth subsalicylate, or a proton pump inhibitor are administered to adults only who suffer from ulcer-related symptoms which include severe abdominal pain and nausea. Current treatment using two antibiotics and a proton pump inhibitor is expensive making it difficult to treat those that are most affected (Wong *et al.*, 1999). Also, children 12 years or younger who suffer from *H. pylori* cannot receive the triple treatment as the Food and Drug Administration (FDA) has not approved the triple treatment regimen. The number and frequency of diseases that result from this infection also make it a concern.

Structure and Disease Mechanism

Helicobacter pylori infects the human stomach and duodenum (figures 1-3), areas in which low pH and constant flushing mechanisms provide a harsh environment for bacteria. However, *H. pylori*'s unique structure and biochemistry allow it to persist in the gastric mucosa of a significant percentage of the population. *H. pylori* is a small, Gram negative, spiral-shaped rod bacterium and is incapable of forming spores (Kelly *et al.*,

1994). Sachs et al. (2003) explain that although technically a neutrophile, *H. pylori* can thrive in the acidic stomach of its host because of urease present in its cytoplasm, working in coordination with urea channels located in both membranes of the gram-negative pathogen. In addition, the spiral form and flagella of *H. pylori* enable motility, which aids the bacteria's journey to its site of infection as it must cross the mucus layer in order to attach to gastric epithelial cells. Adhesin proteins found on the outer membrane of *H. pylori* bind human gastric mucosal antigens as well as molecules on the gastric epithelial cells, effectively adhering the pathogen to its host (Odenbreit, 2005).



Figures 1-3: *H. pylori* infection (<http://www.gicare.com/pated/ecdgs30.htm>).

Once bound, *H. pylori* interacts with the host via a number of molecules including VacA and CagA. However, not all strains are capable of producing these molecules, which leads to another key aspect of *H. pylori* morphology: diverse genetic variation within a population. *H. pylori* inhabits the host not as a clonal colony but as a mixture of strains coexisting and capable of rapid gene transfer (Blaser, 1994; Owen, 1995; Suerbaum and Achtman, 2004). Neither VacA nor CagA, which seem to be important

factors in the development of ulcers and gastric cancer, are present in all strains (Blaser and Atherton, 2004).

H. pylori also have certain structures and molecules that do not activate the human immune system as do corresponding features on other bacteria. For example, Toll-like receptors (TLRs) do not recognize *H. pylori* flagella or its highly methylated DNA (Blaser and Atherton, 2004). Also, Helicobacter lipopolysaccharide (LPS) can resemble human self-antigens, helping *H. pylori* camouflage itself while it infects the human stomach (Moran, 1996; Moran et al., 2005).

Previous Vaccines

A variety of vaccines have been created and tested. However, none have proved to be successful. The following section will give a summary of different vaccines that have been tried.

One vaccine that has been attempted was a recombinant vaccine using *Salmonella typhi* to express UreA and UreB, *H. pylori* urease genes (Kreiss, 1996). This vaccine was given to volunteers over a four week period. There were no side effects, but there was also no immune response to urease.

A second type of a recombinant vaccine has also been tested. This used *Salmonella enterica* serovar *typhimurium* as a live vector also expressing urease (DiPetrillo, 1999). This vaccine was meant to elicit a strong mucosal and systemic B cell and T cell response. Vaccinated individuals showed a measurable serum IgG to *Salmonella*, but there was no humoral or mucosal response to *H. pylori* urease. A different vector, *S. enterica* var. *typhi* Ty21, expressing urease was also tried. However, there was no measurable antibody response (Bumann, 2001). Even those that demonstrated an antibody reaction had a weak T cell response.

Another vaccine containing inactivated whole killed cells plus an adjuvant was administered to a group of volunteers that were uninfected and a group of infected individuals with *H. pylori* (Kotloff, 2001). This trial vaccine contained formalin killed *H. pylori* cells with varying doses of an adjuvant, LT_{R192G}, which is a mutant *E. coli* heat-labile enterotoxin (LT) that contained a glycine instead of an arginine residue in the one of the LT subunits. Unfortunately, this vaccine was not well tolerated as some of the participants experienced adverse side effects. The vaccine raised IFN- γ levels, but it did not raise levels in infected individuals. When this vaccine was given orally *H. pylori*-specific antibody-secreting cells were induced in gastric tissues of uninfected volunteers with a high response in the duodenum (Losonsky, 2003).

There has been work done on a promising new vaccine that includes aluminum hydroxide as an adjuvant with a combination of three purified *H. pylori* antigens (CagA, VacA, and NAP). This has elicited an antibody response after several months from the last immunization (Ruggiero, 2003). Production of IFN- γ did occur and it has been found to be safe. However, specific details have yet to be made available.

Vaccine

Description

This vaccine is a recombinant vector vaccine. Three antigens have been chosen to be expressed by the vector. The first antigen is the protein FlgK. FlgK is a recently

discovered flagellar protein. Flagella are long filaments used by bacteria for motility. These structures can also be used for attachment to host cells. The long filament of a flagellum is attached to a motor by a hook. FlgK is a hook protein found in *H. pylori*. It was discovered FlgK is essential for motility and plays a role in colonization (Wu, 2006). FlgK was chosen as an antigen for this vaccine for a couple of reasons. One reason is that it has not been tested in a vaccine thus far. Also, an antibody response to this protein would prevent *H. pylori* motility and colonization through neutralization.

Another antigen that has been chosen is omp26. Recent studies have discovered a variety of outer membrane proteins (OMP) and compared the differences between disease causing strains and asymptomatic strains (Carlsohn, 2006). This group found omp26 was expressed by all disease-causing strains, but only one in five of the asymptomatic strains exhibited expression. For this reason, we chose omp26 to be another antigen expressed in our vector system.

The last antigen to be expressed by the vector is CagA. The cytotoxin-associated gene A (CagA) is part of the cag pathogenicity island (cagPAI), which is found in disease-causing strains of *H. pylori* (Censini et al., 1996). Once injected into gastric epithelial cells by the bacteria, CagA can alter the host cells' structure, cycle, cytokine release, and gene expression (Hatakeyama and Higashi, 2005), as well as potentially result in oxidative stress to the gastric epithelium (Handa et al., 2007). Concurrent with the speed at which *H. pylori* mutates, CagA protein exists in many different forms based on the population the pathogen is infecting; the main forms are East-Asian CagA and Western CagA (Hatakeyama, 2004), with the East-Asian form being associated with increased virulence. Both forms of CagA will be included in the vaccine. Inactivating CagA through the production of antibodies is an essential function of vaccination in that it would result in decreased *H. pylori*-related gastric carcinogenesis as well as chronic gastritis.

A recombinant vector vaccine was chosen because it is an effective way to get the antigens presented to the mucosal membranes. This type of vaccine has a number of advantages such as a specific vector that will elicit the correct response and is easily cultured, and that desired antigens can easily be purified and expressed. Also, a number of other vaccines that have been tried were unsuccessful. This vaccine will utilize a live *Salmonella typhi* vector. This vector was chosen because *S. typhi* naturally infects the gut. Due to this natural habitat *S. typhi* should help to stimulate mucosal IgA immunity. Also, it has been shown that *Salmonella* is a successful vector for *H. pylori* vaccines (Nardelli-Haeffliger et al., 1996). Also, the use of a live bacterial vector will stimulate the immune system in ways that the antigens alone cannot, and will therefore take the place of an adjuvant.

Immune Response

Essentially, the immune response hoped for will be the same for all three antigens. The reason for having three is to increase chances of eliciting a sufficient memory response to all disease-causing strains of *H. pylori*. Initially, the vaccine will elicit the innate immunity. Macrophages will be the first to encounter the antigens. Macrophage secretion of IL-8 signals neutrophils to the infection site and secretion of IL-1 and IL-6 recruit dendritic cells and other phagocytes to the spot of infection.

Phagocytosis is accomplished primarily by dendritic cells, macrophages, neutrophils, and B cells. The dendritic cells are the primary antigen-presenting cells because of their role in activating T cells. The cytokine TNF- α triggers APCs to migrate to Peyer's patches to meet naïve T cells, to which they present antigen on MHC II. Th0 cells are activated via MHC II bound to CD4, and co-stimulation is accomplished with binding of B7 on the APC to CD28 on the T cell. At this point, the T cell expresses CD40L, a better co-stimulatory molecule than B7, which binds CD40 on the APC. Also, CTLA-4 is expressed and binds stronger to B7. Th0 cells secrete IL-2, IL-4, and IFN- γ . These cytokines are what signal differentiation to Th1 and Th2 T helper cells. The mechanism of differentiation to Th1 or Th2 is not well understood, but it is determined by cytokines, co-stimulation, and the nature of the MHC:peptide presented.

The now-activated Th2 cells work to activate B cells. Cross-linking of BCR must occur in order for activation to be carried out. Antigen is also processed and presented on naïve B cells on MHC II. This MHC II binds Th2 with CD4, as well as CD40L and CD40 bind. Once bound, cytokines IL-4, 5, 6 signal B cell proliferation and differentiation into either plasma cells or memory B cells. Memory B cells make stimulation fast and easy if infected. This occurs in special areas called germinal centers. The plasma cells secrete IgM first, until isotype switching occurs. In the mucosal lining, IgA will be produced because IL-5 stimulates isotype switching to IgA. Th1 cells can also produce the same cytokines that influence isotype switching to IgA. Once activated, most B cells reside in the primary follicle. IgA is primarily used for neutralization of antigen on the mucosal surfaces of the gastrointestinal tract, blocking entry and attachment of the pathogen to host cells.

Humoral immunity will play the major role in this vaccination due to the extracellular nature of *H. pylori* and the need for neutralizing antibodies. Antibodies against each antigen will allow for neutralization and opsonization of the pathogen. IgA will be the main isotype produced since it is a mucosal infection. IgA against FlgK is important to inhibit pathogen motility and adhesion. Omp26 antibodies will also prevent *H. pylori* from binding to host cells. IgG will also play a role in immunity, especially if the pathogen is not neutralized or opsonized before it is able to breach the epithelial lining of the gastrointestinal tract. This isotype will allow neutralization of the toxin CagA.

Concerns

The major concern with this vaccine is the balance between Th1 and Th2 responses. Th2 responses are needed for sufficient antibody production. However, Th1 responses are seen to occur more often in natural cases of *H. pylori* infections (Svennerholm and Lundgren, 2007).

Current trial HIV vaccines are being tested using cytokines as adjuvants (Lori, 2006). This idea may give an answer to the Th1/Th2 balance problem. *H. pylori* vaccine may need to have cytokines as adjuvants to enhance the Th2 response. IL-4 would be the cytokine of choice because it supports differentiation of Th0 cells into Th2 cells. Th2 cells also produce IL-4 to amplify differentiation of Th0 in Th2 and support B cell proliferation and differentiation. Th2 cells will also secrete IL-10, which will suppress Th1 cells, and therefore promote the desired response of antibody production, rather than

the ineffective Th1 response which is naturally induced by and dominates *H. pylori* infection.

Vaccine Trial

Administration and Trials

This vaccine will be administered orally for a variety of reasons. These reasons include cost, ease of administration, ease of storage, direct targeting to mucosal membranes, and previous work done. The first three reasons will be especially important considering the high risk groups of *H. pylori* infection are most abundant in developing countries.

Testing in mice and possibly higher mammals will begin our vaccine trials in order to verify that an immune response does occur, and to test for the appropriate dosage. After animal testing, clinical trials can begin. There are three phases to clinical trials. Phase I is performed on a small group of about 20 healthy, non-infected, low-risk volunteers. Four groups of five people will be given different doses of vaccine. The first group will get 20 mg, the second group 40 mg, the third group 80 mg, and the final group 160 mg. Immunity will be tested at weeks one, two, four, and eight after administration. Based on the results generated, Phase I will determine the amount and schedule of the vaccine to be given in subsequent trials.

Phase II consists of a larger group of people (50-100). These are also healthy, uninfected volunteers. The purpose of this phase is to refine dosage and schedule using dosage that was previously found to work and varying closer to that range. After analyzing the results of immunity testing in Phase I, any unsuccessful doses will be eliminated, and the need for booster dosing will be looked into and possibly put into effect for Phase II. Immune responses will be tested at 2, 4 and 6 months after administration. These results will be further refined for the planning of Phase III.

Phase III is the final stage of clinical testing, and therefore the most crucial to getting the vaccine approved for use in the general population. This is a large scale (500-1000 people) trial on uninfected, high risk volunteers. High risk will be determined by the prevalence of *H. pylori* infection in the area the volunteer is from. Phase III will be a double-blind study consisting of a control group receiving a placebo, and a test group which receives the vaccine. The dosage and schedule found to be most effective in Phase II will be what is given to participants in Phase III.

Testing for Immunity

To check for humoral immunity an ELISA will be performed. Plastic wells are coated with antigen. In this case, there are three different antigens, FlgK, omp26, and CagA. These antigens will have to be tested in three separate tests. The primary antibody added is taken from the immunized patient. Then a secondary antibody specific to human IgA conjugated to an enzyme is added. The presence of IgA bound to antigen will be shown by a change in color. This is a highly specific and sensitive test to check for humoral immunity. This test will also show which antigens elicited the best response. Other vaccine attempts could benefit from knowing the best antigens to target.

Testing for cell-mediated immunity is done with an ELISPOT. Plastic wells are coated with cytokine-specific antibodies. Th2 cells taken from immunized patients are

then added. The cytokines released become bound to the antibodies. A second cytokine specific antibody that is enzyme linked is then added to the well. A colored spot will then show where the cytokines were secreted. IL-4, IL-5, and IL-6 will be the cytokines looked for because they are Th2 specific.

References

1. Blaser MJ. *Helicobacter pylori* phenotypes associated with peptic ulceration. **Scand J Gastroenterol Suppl**, **205**: 1-5, 1994.
2. Blaser MJ, Atherton JC. *Helicobacter pylori* persistence: biology and disease. **J Clin Invest**, **113**: 321-33, 2004.
3. Bumann D, Metzger WG, Mansouri E, Palme O, Wendland M, Hurwitz R, Haas G, Aebischer T, von Specht BU, Meyer TF. Safety and immunogenicity of live recombinant *Salmonella enteric* serovar *typhi* Ty21a expressing urease A and B from *Helicobacter pylori* in human volunteers. **Vaccine**, **20**: 845-52, 2001.
4. Carlsohn, E, Nystrom, J, Karlsson, H, Svennerholm, AM, Nilsson, CL. Characterization of the outer membrane protein profile from disease-related *Helicobacter pylori* isolates by subcellular fractionation and nano-LCFT-ICR MS analysis. **Journal of Proteome Research**, **5**: 3197-204, 2006.
5. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. Cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. **Proc Natl Acad Sci USA**, **93**: 14648-53, 1996.
6. DiPetrillo MD, Tibbetts T, Kleanthous H, Killeen KP. Safety and immunogenicity of phoP/phoQ-deleted *Salmonella typhi* expressing *Helicobacter pylori* urease in adult volunteers. **Vaccine**, **18**: 449-59, 1999.
7. Handa O, Naito Y, Yoshikawa T. CagA protein of *Helicobacter pylori*: a hijacker of gastric epithelial cell signaling. **Biochem Pharmacol**, **73**: 1697-702, 2007.
8. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. **Nat Rev**, **4**:688-94, 2004.
9. Hatakeyama M, Higashi H. *Helicobacter pylori* CagA: a new paradigm for bacterial carcinogenesis. **Cancer Sci**, **96**: 835-43, 2005.
10. Kelly SM, Pitcher MC, Farmery SM, Gibson GR. Isolation of *Helicobacter pylori* from feces of patients with dyspepsia in the United Kingdom. **Gastroenterol**, **107**:16771-4, 1994.

11. Koletzko S, Richy F, Bontems P et al., Prospective multicentre study on antibiotic resistance of *Helicobacter pylori* strains obtained from children living in Europe. **Gut**, **55**:1711-16, 2006.
12. Kotloff KL, Sztein MB, Wasserman SS, Losonsky GA, DiLorenzo SC, Walker RI. Safety and immunogenicity of oral inactivated whole-cell *Helicobacter pylori* vaccine with adjuvant among volunteers with or without subclinical infection. **Infect Immun**, **69**: 3581-90, 2001.
13. Kreiss C., Buclin T, Cosma M, Corthesy-Theulaz I, Michetti P. Safety of oral immunization with recombination urease in patients with *Helicobacter pylori* infection. **Lancet**, **347**: 1630-1, 1996.
14. Lori F, Weiner DB, Calarota SA, Kelly LM, Lisciewicz J. Cytokine-adjuvanted HIV-DNA vaccination strategies. **Springer Semin Immunopathol**, **28**: 231-8, 2006.
15. Losonsky GA, Kotloff KL, Walker RI. B-cell responses in gastric antrum and duodenum following oral-inactivated *Helicobacter pylori* whole-cell (HWC) vaccine and LT (R192G) in *H. pylori*-seronegative individuals. **Vaccine**, **2**: 562-5, 2003.
16. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. **Lancet**, **1**: 1311-15, 1984.
17. Moran AP. Bacterial surface structures—an update. **FEMS Immunol Med Microbiol**, **16**: 61-2, 1996.
18. Moran AP, Khamri W, Walker MM, Thursz MR. Role of surfactant protein D (SP-D) in innate immunity in the gastric mucosa: evidence of interaction with *Helicobacter pylori* lipopolysaccharide. **J Endotoxin Res**, **11**: 357-62, 2005.
19. Nardelli-Haeffliger D, Kraehenbuhl JP, Curtiss R 3rd, Schodel F, Potts A, Kelly S, De Grandi P. Oral and rectal immunization of adult female volunteers with a recombinant attenuated *Salmonella typhi* vaccine strain. **Infect Immun**, **64**: 5219-24, 1996.
20. Odenbreit S. Adherence properties of *Helicobacter pylori*: impact on pathogenesis and adaptation to the host. **Int J Med Microbiol**, **295**:317-24, 2005.
21. Owen RJ. Bacteriology of *Helicobacter pylori*. **Baillieres Clin Gastroenterol**, **9**:415-46, 1995.
22. Ruggiero P, Peppoloni S, Rappuoli R, Del Giudice G. The quest for a vaccine against *Helicobacter pylori*: how to move from mouse to man? **Microbes Infect**, **5**: 749-56, 2003.

23. Sachs G, Weeks DL, Melchers K, Scott DR. The gastric biology of *Helicobacter pylori*. **Annu Rev Physiol**, **65**:349-69, 2003.
24. Sonnenberg, A. Time Trends of Ulcer Mortality in Non-European Countries. **Am J Gastroenterol**, **102**: 1101-7, 2007.
25. Suerbaum S, Achtman M. *Helicobacter pylori*: recombination, population structure and human migrations. **Int J Med Microbiol**, **294**:133-9, 2004.
26. Svennerholm AM, Lundgren A. Progress in vaccine development against *Helicobacter pylori*. **FEMS Immunol Med Microbiol**, **50**: 146-56, 2007.
27. Wong BC, Lam SK, Lai KC, Hu WH, Ching CK, Ho J, Yuen ST, Chan CK, Lau GK & Lai CL. Triple therapy for *Helicobacter pylori* eradication is more effective than long-term maintenance antisecretory treatment in the prevention of recurrence of duodenal ulcer: a prospective long-term followup study. **Aliment Pharmacol Ther** **13**: 303-9, 1999.
28. Wu JJ, Sheu BS, Huang AH, Lin ST, Yang HB. Characterization of flgK gene and FlgK protein required for *H. pylori* colonization—from cloning to clinical relevance. **World J Gastroenterol**, **12**: 3989-93, 2006.