Published in final edited form as:
Vaccine. 2011 March 3; 29(11): 2085–2091. doi:10.1016/j.vaccine.2010.12.130.

HelicoVax: Epitope-based therapeutic *H. pylori* vaccination in a mouse model

Steven F. Moss[1,*], **Leonard Moise**[2,3], **Dong Soo Lee**[1], **Woojin Kim**[1], **Songhua Zhang**[1], **Jinhee Lee**[4], **Arlin B. Rogers**[5], **William Martin**[2], and **Anne S. De Groot**[2,3,6]

[1] Department of Medicine, Division of Gastroenterology, Rhode Island Hospital & Warren Alpert Medical School of Brown University, Providence, Rhode Island [2] EpiVax, Inc., Providence, Rhode Island [3] Institute for Immunology and Informatics, University of Rhode Island, Providence, Rhode Island [4] University of Massachusetts Medical School, Worcester, Massachusetts [5] Department of Pathology, University of North Carolina, Chapel Hill, North Carolina [6] Department of Pediatric Infectious Disease, Brown University Warren Alpert Medical School, Providence, Rhode Island

Abstract

Helicobacter pylori is the leading cause of gastritis, peptic ulcer disease and gastric adenocarcinoma and lymphoma in humans. Due to the decreasing efficacy of anti-*H. pylori* antibiotic therapy in clinical practice, there is renewed interest in the development of anti-*H. pylori* vaccines. In this study an in silico-based approach was utilized to develop a multi-epitope DNA-prime/peptide-boost immunization strategy using informatics tools. The efficacy of this construct was then assessed as a therapeutic vaccine in a mouse model of gastric cancer induced by chronic *H. pylori* infection. The multi-epitope vaccine administered intranasally induced a broad immune response as determined by interferon-gamma production in ELISpot assays. This was associated with a significant reduction in *H. pylori* colonization compared with mice immunized with the same vaccine intramuscularly, given an empty plasmid, or given a whole *H. pylori* lysate intranasally as the immunogen. Total scores of gastric histological changes were not significantly different among the 4 experimental groups. These results suggest that further development of an epitope-based mucosal vaccine may be beneficial in eradicating *H. pylori* and reducing the burden of the associated gastric diseases in humans.

[*]**Correspondence to:** Steven F. Moss, M.D., Gastroenterology Division, Rhode Island Hospital, 593 Eddy Street, APC 414, Providence, RI 02903. Tel: (401) 444-6713; Fax: (401) 444-2939. Steven_Moss@Brown.edu.
Current affiliations: Woojin Kim: Gastroenterology Division, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA
Dong Soo Lee: Daejeon St. Mary's Hospital, Catholic University of Korea, Daejeon, Republic of Korea

Two of the contributing authors, Anne S. De Groot and William D. Martin are senior officers and majority shareholders at EpiVax, Inc., a privately-owned vaccine design company located in Providence, RI. Dr. De Groot is also a faculty member at the University of Rhode Island and Brown University.

Conflict of interest statement

These authors acknowledge that there is a potential conflict of interest related to their relationship with EpiVax and attest that the work contained in this research report is free of any bias that might be associated with the commercial goals of the company.

Introduction

Helicobacter pylori represents a significant public health challenge since it causes chronic gastritis and significantly increases the risk of developing peptic ulcer disease and gastric cancer [1]. *H. pylori* infection is highly prevalent, particularly in developing countries. The strong epidemiological linkage between *H. pylori* and distal (non-cardia) cancer led the World Health Organization's International Agency for Cancer Research branch to classify *H. pylori* as a definite (type I) carcinogen in 1994 [2], a conclusion confirmed by the same agency last year [3].

The co-evolution of *H. pylori* with human beings over tens of thousands of years and the inverse relationship between *H. pylori* and certain conditions, such as gastroesophageal reflux disease, has led to the suggestion that persistent *H. pylori* infection may provide some benefit to humanity, especially in the developed world [4]. Nevertheless, for most infected individuals the balance of evidence supports eradicating *H. pylori* to prevent gastritis, ulcer disease and gastric cancer, especially where *H. pylori* and gastric cancer are very common, such as in the Asian-Pacific region [5].

Current treatments to eradicate *H. pylori* involve the use of multiple antibiotics in combination with acid suppression medication over 1-2 weeks, an approach that has changed little over the last 3 decades since *H. pylori* was first identified [1]. Because of antibiotic resistance (especially to metronidazole and clarithromycin), *H. pylori* is more difficult to eradicate now than it had been formerly [6] leading to renewed interest in developing alternative therapeutic strategies such as vaccines [7]. Vaccination against *H. pylori* may be a particularly attractive choice as the most practical way to avoid *H. pylori*-associated gastric cancer in the developing world [8].

In humans, *H. pylori* stimulates an intense gastric inflammatory response that persists over the course of infection that typically lasts many decades. Animal models have established that the pathogenesis of gastric diseases induced by *H. pylori* is related to the Th1-skewed T-cell response and that humoral immunity does not provide protection against infection. Despite the generation of robust cellular and humoral immune responses, spontaneous eradication of the organism is extremely rare in humans [1], likely related to several properties of the bacterium that help it evade natural immune responses and allow persistence [4]. While normal immune mechanisms fail to eradicate *H. pylori*, some prophylactic and therapeutic vaccines strategies using a wide variety of *H. pylori* antigens have been reported to successfully eradicate *H. pylori* from mice and in a few studies in human volunteers [7]. Nevertheless the correlates of gastric immunity to *H. pylori* are not well defined and the optimal choice of antigen and best delivery method for further clinical development remain to be determined.

In order to advance our *H. pylori* vaccine strategy we used a gene-to-vaccine approach, incorporating multiple epitopes in a DNA-prime/peptide boost approach as previously described [9], using informatics tools that have been successfully applied to AIDS, TB, smallpox and tularemia vaccine development [9-13]. We present our initial findings on the efficacy of this construct as a therapeutic vaccine in a mouse model of gastric cancer induced by chronic *H. pylori* infection. Our results suggest that a broad-spectrum epitope-driven immune response against *H. pylori* antigens can lead to successful eradication of the organism.

Methods

Mice, *H. pylori* infection and overall experimental plan

A total of 80 male and female C57BL/6 mice deficient in the *CDKN1B* gene encoding p27 were used in this study. Because female heterozygous *CDKN1B*-deficient mice are sterile, the mice for this study were derived from breeding male homozygous deficient to female heterozygotes and then identifying homozygous deficient pups by genotyping. Heterozygote founders of this colony had been originally purchased from Jackson laboratories (Jackson Labs, Bar Harbor, ME). All studies were performed in full compliance with the standards of the Rhode Island Hospital Institutional Animal Care and Use Committee and in accordance with NIH publications entitled “Principles for Use of Animals” and “Guide for the Care and Use of Laboratory Animals.”

At 6 weeks of age mice were infected with 10^9 *H. pylori* strain SS1 in 0.1 mL PBS by gavage on 3 occasions over 1 week using a balled animal feeding needle as previously described [14].

There were 4 experimental groups (20 mice per group); the experimental design is shown in Figure 1. At weeks 11-15 of age, mice were given doses of a *H. pylori* SS1 lysate, an empty plasmid (“pVAX control”) or a DNA based vaccine intramuscularly or intranasally. At ages 17 and 18 weeks mice were given a boost of intranasal peptides formulated in liposomes (or empty liposome as a control).

All mice were maintained in micro-isolator cages to reduce inter-cage *Helicobacter* infection and allowed access to Harlan Teklad Global Diet 2018 (Harlan, Indianapolis, IN) and water ad libitum, in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of Rhode Island Hospital. At 51 weeks of age (45 weeks after *H. pylori* infection) mice were euthanized by cervical dislocation under isoflurane anesthesia for bacteriological, immunological and histological analyses.

Immunizations

A lysate of *H. pylori* strain SS1 was prepared by French press and clarified by centrifugation as described previously [15]. 200 microgram of *H. pylori* lysate with 10 microgram *E. coli* heat-labile LT (R192G) adjuvant in 20 microliters PBS was administered intranasally once weekly for 5 weeks. [16].

In the group receiving pVAX control intranasally, 100 micrograms of an empty plasmid was given with 10 microgram LT (R192G) adjuvant in 20 microliters PBS on 3 occasions over 5 weeks (weeks 11,13 and15), and 20 microliters PBS only was given intranasally on two occasions (weeks 12 and 14).

In the group receiving DNA vaccine intramuscularly, 100 micrograms of DNA in 0.1 milliliter PBS was given into the flank on 3 occasions over 5 weeks (weeks 11,13 and 15) and 20 microliters PBS only was given intranasally on two occasions (weeks 12 and 14).

In the group receiving DNA vaccine intranasally, 100 micrograms of DNA was given with 10 microgram LT (R192G) adjuvant in 20 microliters PBS on 3 occasions over 5 weeks (weeks 11,13 and 15) and 20 microliters PBS only was given intranasally on two occasions (weeks 12 and 14).

To boost responses in the two DNA-based vaccine arms, a 10 microliter volume of liposomal peptide vaccines (50 microgram) with 20 microgram immunostimulatory CpG oligonucleotides and 10 microgram LT (R192G) were given intranasally at week 17 and at

week 19. All peptides were formulated together in a single preparation so that all the mice treated with peptides receive the identical peptide combination. The other two groups of mice (*H. pylori* lysate, pVax control) were given empty liposomes instead, at the same timepoints.

Multi-epitope DNA vaccine engineering

Epitope sequences were concatenated to form two multi-epitope genes (termed HelicoVax A and HelicoVax B), each containing 25 HLA Class II epitopes that were identified by immunoinformatics methods, as described previously [17]. The detailed final composition and epitope order are listed in Supplemental Table 1 and Supplemental Table 2. All mice in the DNA vaccine groups received both HelicoVax A and HelicoVax B. Initially, epitopes were assembled in a random sequence. To avoid creation of novel epitopes at epitope junctions, an algorithm which iteratively re-orders epitopes to reduce junctional immunogenicity (VaccineCAD) was used to optimize epitope order [10]. In addition, Gly-Pro-Gly-Pro-Gly spacer sequences were engineered between some epitopes to optimize epitope processing, where re-ordering by VaccineCAD did not sufficiently reduce potential junctional immunogenicity [18].

A Kozak sequence was engineered upstream of the coding sequence for efficient translation initiation. To target the immunogens to the Class II processing pathway, the tissue plasminogen activator (tPA) leader sequence (MQMSPALTCLVLGLALVFGEGSA) was placed upstream of epitope sequences to direct translation products to the secretory pathway. A histidine tag was incorporated downstream of the epitope sequences followed by two stop codons. Genes were synthesized by GeneArt (Burlingame, CA) and subcloned at pre-determined flanking restriction sites into pVAX1 (Invitrogen, Carlsbad, CA), a DNA vaccine vector that accommodates FDA recommendations for construction of plasmid DNA vaccines [19]. High purity plasmids for immunizations were prepared by PureSyn, Inc. (Malvern, PA) at pre-clinical grade.

Peptide vaccine preparation

Peptides were manufactured using 9-fluoronylmethoxycarbonyl (Fmoc) chemistry by New England Peptide (Gardner, MA). Master batch records indicate that peptides were purified to >80% as ascertained by analytical reversed phase HPLC and peptide mass was confirmed by MALDI-TOF mass spectrometry.

The constituent peptides of the DNA vaccine were formulated in liposomes with immunostimulatory CpG oligodeoxynucleotide (ODN) 1555 (5′-GCTAGACGTTAGCGT-3′; Integrated DNA Technologies, Coralville, IA) [20] and heat-labile enterotoxin, LT (R192G), kindly provided by Dr. John Clements (Tulane University). Sterically stable cationic liposomes were prepared from three lipid components: 11.9 μmol dioleylphosphatidylethanolamine, 7.9 μmol dimethylaminoethanecarbamol-cholesterol, and 1.1 μmol polyethylene glycol 2000-phosphatidyl-ethanolamine (Avanti Polar Lipids, Alabaster, AL). The lipids were mixed, dried in a rotary evaporator and re-suspended in PBS to make empty multi-lamellar vesicles. These vesicles were then sonicated for 2 min to convert them into unilamellar liposomes. Unilamellar liposomes (10 μmol) were mixed with CpG ODN (400 μg) and peptides (1 mg), flash frozen and freeze-dried overnight. To encapsulate CpG ODN and peptides in liposomes, the resulting powder was re-suspended with sterile distilled water and vortexed for 15 seconds every five minutes for 30 minutes at room temperature. PBS and LT (R192G) were added to yield the final required vaccine volume and LT dose, as described above. Liposome formulations were stored at 4°C until use.

H. pylori quantification

H. pylori infection was quantified by real-time PCR of the *SSA* gene of *H. pylori*, normalized for mouse stomach GAPDH expression, as previously described [14].

Gastric histology

Gastric histology was evaluated without knowledge of experimental group in H&E-stained sections and scored for epithelial and inflammatory cell parameters using the criteria and grading system of Rogers et al [21].

ELISpot assay

The frequency of epitope-specific splenocytes was determined by IFN-gamma ELISpot assay using the Mabtech IFN-gamma ELISpot Kit according to the manufacturer's protocol (Mariemont, OH). Briefly, spleens were harvested from groups of control and vaccinated mice and mascerated to produce single cell splenocyte suspensions in RPMI-10% fetal bovine serum-1% penicillin/streptomycin-1% L-glutamine-0.1% BME at a concentration of 3×10^6 cells/ml. Cells were transferred at 3×10^5/well to ELISpot plates pre-coated with anti-murine IFN-gamma by the manufacturer and stimulated with 50 individual and 6 pools of 5-10 peptides at 10 μg/ml in triplicate wells. Pools 1 to 3 correspond to the epitopes contained in DNA vaccine construct A (HelicoVaxA) and pools 4 to 6 correspond to epitopes present in DNA vaccine construct B (HelicoVaxB). Positive controls included cells stimulated with Con A (2 μg/ml) or SS1 lysate (10 μg/ml); no-peptide stimulation served as a negative control. ELISpot plates were incubated at 37°C, 5% CO_2 for 2 days, washed, incubated with a secondary HRP labeled anti-IFN-gamma antibody and developed by addition of TMB substrate. Spot counts were determined by Zellnet, Inc. using a Zeiss ELISpot plate reader. Results were recorded as the average number of spots over background and adjusted to spots per one million cells seeded. Responses are considered positive if the number of spots is: 1) at least twice greater than background, 2) greater than 50 spots forming cells per one million splenocytes over background (i.e. one response over background per 20,000 splenocytes), and 3) statistically significant by Student's t-test in comparison with the corresponding spot forming cell data set for other groups ($p<0.05$).

Statistics

Differences between experimental groups were compared by one-way analysis of variance with Dunnett's multiple comparison test, or the non-parametric equivalent, Kruskal-Wallis with Dunn's multiple comparison test, as appropriate (GraphPad Prism version 5.01, GraphPad, La Jolla, CA).

Results

Multi-epitope DNA vaccine construction

We designed two DNA vaccines, HelicoVax A and HelicoVax B, each of which contains a distinct set of 25 HLA class II epitopes (Figure 2 and Table 1). The epitopes were previously identified using immunoinformatics methods that selected immunogenic and conserved sequences from the *H. pylori* 26695 and J99 genomes [17]. Epitope sequences were randomly concatemerized, at first. To avoid production of neo-epitopes at epitope junctions, the VaccineCAD algorithm was used to re-arrange epitopes in an order that diminishes potential junctional immunogenicity. The default order for each construct contained significant predicted immunogenicity at two junctions in HelicoVax A and none in HelicoVax B (EpiMatrix scores >10). Re-ordering of epitopes by VaccineCAD and insertion of GPGPG spacers (24) produced sequences with minimized junctional immunogenicity (EpiMatrix scores < 4.5) (Supplemental Data Tables 1 and 2).

H. pylori colonization

A small number of mice died due to peri-procedural complications, so that the final numbers of mice available at study end were 18 in the lysate and DNA IM groups, 17 in the empty plasmid control group and 19 in the DNA IN group.

The results of *H pylori* infection density evaluated by real-time PCR are shown in Figure 3. Mice in the DNA IN group had the lowest numbers of bacteria, with 5 of the 19 mice having no detectable *H. pylori* DNA (compared with 2 of 17 in the pVAX group, none of 18 in the lysate group and 3 of 18 in the DNA IM group). Considerable variation in infection density within each group was evident, but the median value for the DNA IN group (1.05) was significantly lower than the median for each of the other 3 groups, lysate median 47.4, pVAX median 25, DNA IM median 53.4 ($P<0.001$ versus lysate, $P<0.01$ versus pVAX, and $P<0.001$ versus DNA IM).

Histology

Histological abnormalities in these mice are shown in Table 2, including the median (range) scores for inflammation, epithelial defects, oxyntic atrophy, hyperplasia, metaplasia and dysplasia, as well as total score. Overall, the histological changes were quite mild and differences were only statistically significant for inflammation and dysplasia, when comparing mice treated with pVAX versus lysate (less advanced histological changes in the pVAX group, $P<0.05$ for inflammation, $P<0.01$ for dysplasia).

Immune responses

ELISpot assays of epitope-stimulated splenocytes in the DNA IN and DNA IM groups demonstrated that 33/50 epitopes (66%) stimulated >50 interferon-gamma spot forming cells per million splenocytes (Figure 4, gray bars), compared to only 2/50 epitopes (4%) which were recognized in SS1 lysate-immunized animals (Figure 4, open bars). Additionally, all peptide pools stimulated robust interferon-gamma production. There was no consistent difference between intranasal (filled circles) and intramuscular (open circles) DNA immunization on the single-epitope level, but intranasal vaccination generally was associated with stronger responses, particularly among the most immunogenic epitopes. Consistent with the colonization data, SS1 lysate and DNA IM groups, respectively, stimulated 2 and 6 statistically significant responses when compared to the pVAX group, while 23 significant responses were observed in the DNA IN group ($P<0.05$). pVAX alone did not stimulate any statistically significant ELISpot responses

Discussion

The results of this study suggest that a DNA-prime/peptide-boost vaccine designed to present multiple *H. pylori* epitopes has therapeutic efficacy when delivered intranasally to mice previously infected by *H. pylori*. The multi-epitope vaccine induced a broad immune response that led to a significant reduction in *H. pylori* colonization. In contrast, mice given a whole *H. pylori* lysate or an empty plasmid intranasally as the immunogen, or immunized with the same vaccine intramuscularly failed to eradicate the organism. These encouraging, though preliminary, results suggest that further development of an epitope-based mucosal vaccine against *H. pylori* can potentially lead to a novel approach to prevent *H. pylori*-associated diseases such as peptic ulcer disease and gastric cancer in humans.

Importantly, the vaccine-induced immune response parallels what is observed in natural infection. *H. pylori* infection dramatically influences the gastric leukocyte environment with recruitment of T cell populations, primarily the $CD4^+$ phenotype [22], that constitute an enriched source (~1-10%) of *H. pylori*-specific T cells compared with peripheral T

lymphocytes [23,24]. Gastric T cells isolated from biopsies of *H. pylori*-infected subjects exhibit a predominantly proinflammatory Th1 phenotype characterized by a high frequency of IFN-γ-secreting cells [22,25]. The finding that live *H. pylori* preferentially stimulates production of IL-12 [26], which selects for Th1 cell development, may explain the finding that the majority of Th cell clones derived from *H. pylori*-infected patients are comprised of Th1 effector cells [23,27]. *H. pylori* pathogenesis also involves regulatory T cells (Tregs). Depletion of $CD4^+/CD25^{hi}$ Tregs from infected human donors leads to increased *H. pylori*-specific memory T cell proliferation and IFN-gamma production in response to *H. pylori* pulsed dendritic cells. Additionally, increased levels of $CD4^+/CD25^{hi}/FoxP3^+$ regulatory T cells have been observed in gastric and duodenal mucosa of *H. pylori*-infected humans [28]. Moreover, similar results have been described in mouse models of *H. pylori* infection [29,30]. These findings underscore the importance of a Treg response in *H. pylori* immunopathogenesis and suggest that persistence of *H. pylori* infection may be explained by Treg-mediated suppression of pathogen-specific memory T-cells [31]. As Tregs contribute to equilibrium between host and pathogen, inflammation is maintained at low levels, allowing *H. pylori* to survive. Taken together, immunopathogenesis studies suggest that a vaccine that elicits a strong Th1 response, like the $CD4^+$ T-cell epitope-based vaccine described here, should support the immune system's fight against *H. pylori* infection. However, we appreciate that identifying correlates of vaccine efficacy against *H. pylori* remains a major challenge [7].

The CDKN1B deficient mouse model was chosen for this study because it had been shown to develop gastric cancer about 1 year after *H. pylori* infection, following a sequence of progressive histological changes (gastritis, glandular atrophy, metaplasia and dysplasia) similar to the events that appear during gastric carcinogenesis in humans infected by *H. pylori* [14]. One of the aims of this study was to determine whether the therapeutic vaccine could inhibit these progressive histological changes and thus prevent cancer in this model. However histological changes in all four arms of this study were relatively mild, even in the group that received no immunogen (the pVAX group). Indeed, histological abnormalities evaluated at 45 week post-infection were, if anything, less severe in those mice that received the pVAX construct than in mice that had been exposed to *H. pylori* lysate or the epitope based *H. pylori* DNA vaccines. Although it might be assumed that reduction in *H. pylori* colonization will lead to less inflammation and other features of gastric damage included in the histological score, it is important to note that within groups of C57BL/6 mice successfully infected by the SS1 *H. pylori* strain, there is an inverse relationship between the bacterial load and the total gastritis score [32]. Another important consideration when interpreting histological changes is the phenomenon of “post immunization gastritis” in which successful elimination of *H. pylori* following vaccination is accompanied by a transient increase of gastritis over a few weeks, though this ultimately abates with time [33]. The current study evaluated inflammation only at the end of the experiment and therefore the kinetic changes in gastritis over the course of the 45 week infection period remains unknown; it is conceivable that post-immunization gastritis (presumably absent in the pVAX group) might account for some of the discrepancy between the histological scores and the *H. pylori* colonization at 45 weeks post infection.

A limitation of the current study is that we did not include a group of mice that were infected with *H.pylori* but were not subsequently subject to any subsequent experimental manipulations at all (a true “negative control” group). Thus, we cannot state with certainty that the DNA IM strategy or the HP lysate strategy (or indeed the pVAX control) did not reduce *H.pylori* colonization. We can only state with certainty that the DNA IN strategy was superior to the other 3 strategies. Indeed, because all mice in the study received LT as adjuvant, a non-specific effect of adjuvant also cannot be entirely ruled out. We suspect that the intranasal route to deliver the epitope-based DNA vaccine was more efficacious than the

identical vaccine delivered intramuscularly because mucosal immunization likely produced a more robust gastric mucosal cell-mediated immune response against *H.pylori*. However, further studies will be necessary to directly test this hypothesis.

Despite decades of research, the immune response to *H. pylori* and, in particular, the correlates of successful immunity necessary for *H. pylori* vaccination remain poorly understood. Based upon many previous studies in animal models of infection it is known that the production of antibody in the stomach (IgA) provides little or no protection against *H. pylori* infection [34]. In contrast MHC Class II restricted T-cells including Th1, Th2 and Th17 cells are important in mediating immunity in murine models [7]. In humans, chronic infection by *H. pylori* is associated with robust immune and inflammatory responses that nevertheless rarely succeed in eradicating the organism and decades long persistence is typical, related in part to the strategy of *H. pylori* to survive and evade immune defenses [4].

The choice of the CDKN1B mouse as a model for testing the potential therapeutic efficacy of an epitope based vaccine against *H. pylori* was predicated upon increased susceptibility of these mice to gastric cancer and the opportunity to determine whether therapeutic immunization against *H. pylori* could reduce recurrence of this highly clinically significant endpoint. However, the deficiency of p27 in all lineages, including of the lymphoid lineages, may have limited extrapolation from this model to other murine models for *H. pylori* infection. For example, in contrast to wild-type mice the T cells of p27-deficient mice are more susceptible to apoptosis following interleukin-2 withdrawal demonstrating the importance of p27 in the survival of activated T-cells [35]. These mice also have reduced proliferative capacity of their T-cells, especially the CD8+ subset [36]. While p27 deficiency does not apparently affect the differentiation of T cells into Th1 versus Th2 subsets, [37] exaggerated inflammatory responses have been noted in mice populated with p27 deficient bone marrow [38] suggesting that deficiency of p27 in bone marrow-derived progenitor cells can promote inflammatory responses. Furthermore, two groups have demonstrated failure of T-cell tolerance in p27-deficient mice [39,40].

Taken together, these results demonstrate subtle but potentially important abnormalities in the immune system of p27-deficient mice that should be borne in mind when extrapolating from the current results to those that might be expected in wild type mice. Future studies are planned using “humanized” HLA transgenic mice as a bridge to clinical vaccine development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by Public Health Service grants R43AI065036 (ASD), U19AI082642 (ASD) & R01CA111533 (SFM).

We appreciate the assistance of Emilia Mia Sordillo (St. Luke's-Roosevelt Hospital Center, New York, NY) for lysate preparation, John D. Clements (Tulane University, New Orleans, LA) for providing LT (R192G), and Jacques Pappo and Julie McMurry for helpful discussions on study design.

References

1. McColl KE. Clinical practice. *Helicobacter pylori* infection. N Engl J Med 2010;362:1597–604. [PubMed: 20427808]

2. IARC. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994;61:1–241. [PubMed: 7715068]
3. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, Ghissassi F El, BenbrahimTallaa L, Guha N, Freeman C, Galichet L, Cogliano V, WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens--Part B: biological agents. Lancet Oncol 2009;10:321–2. [PubMed: 19350698]
4. Atherton JC, Blaser MJ. Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. J Clin Invest 2009;119:2475–87. [PubMed: 19729845]
5. Fock KM, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, Lam SK, Xiao SD, Tan HJ, Wu CY, Jung HC, Hoang BH, Kachintorn U, Goh KL, Chiba T, Rani AA, Second Asia-Pacific Conference. Second Asia-Pacific Consensus Guidelines for *Helicobacter pylori* infection. J Gastroenterol Hepatol 2009;24:1587–600. [PubMed: 19788600]
6. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut 2010;59:1143–53. [PubMed: 20525969]
7. Velin D, Michetti P. Advances in vaccination against *Helicobacter pylori*. Expert Rev Gastroenterol Hepatol 2010;4:157–66. [PubMed: 20350263]
8. Arora S, Czinn SJ. Vaccination as a method of preventing *Helicobacter pylori*-associated gastric cancer. Cancer Epidem Biomark Prev 2005;14:1890–1.
9. Moise L, Buller RM, Schriewer J, Lee J, Frey SE, Weiner DB, Martin W, De Groot AS. VennVax, a DNA-prime, peptide-boost multi-T-cell epitope poxvirus vaccine, induces protective immunity against vaccinia infection by T cell response alone. Vaccine. Nov 4;2010 doi:10.1016.
10. De Groot AS, Marcon L, Bishop EA, Rivera D, Kutzler M, Weiner DB, Martin W. HIV vaccine development by computer assisted design: the GAIA vaccine. Vaccine 2005;23:2136–48. [PubMed: 15755584]
11. McMurry JA, Kimball S, Lee JH, Rivera D, Martin W, Weiner DB, Kutzler M, Sherman DR, Kornfeld H, De Groot AS. Epitope-driven TB vaccine development: a streamlined approach using immuno-informatics, ELISpot assays, and HLA transgenic mice. Curr Mol Med 2007;7:351–68. [PubMed: 17584075]
12. McMurry JA, Gregory SH, Moise L, Rivera D, Buus S, De Groot AS. Diversity of *Francisella tularensis* Schu4 antigens recognized by T lymphocytes after natural infections in humans: identification of candidate epitopes for inclusion in a rationally designed tularemia vaccine. Vaccine 2007;25:3179–91. [PubMed: 17291638]
13. Gregory SH, Mott S, Phung J, Lee J, Moise L, McMurry JA, Martin W, De Groot AS. Epitope-based vaccination against pneumonic tularemia. Vaccine 2009;27:5299–306. [PubMed: 19616492]
14. Kuzushita N, Rogers NAB, Monti NA, Whary MT, Park MJ, Aswad BI, Shirin H, Koff A, Eguchi H, Moss SF. $p27^{kip1}$ deficiency confers susceptibility to gastric carcinogenesis in *Helicobacter pylori*-infected mice. Gastroenterology 2005;129:1544–56. [PubMed: 16285954]
15. Pappo J, Torrey D, Castriotta L, Savinainen A, Kabok Z, Ibraghimov A. *Helicobacter pylori* infection in immunized mice lacking major histocompatibility complex class I and class II functions. Infect Immun 1999;67:337–41. [PubMed: 9864234]
16. Akhiani AA, Pappo J, Kabok Z, Schön K, Gao W, Franzén LE, Lycke N. Protection against *Helicobacter pylori* infection following immunization is IL-12-dependent and mediated by Th1 cells. J Immunol 2002;169:6977–84. [PubMed: 12471132]
17. Moise L, McMurry JA, Pappo J, Lee DS, Moss SF, Martin WD, De Groot AS. Identification of genome-derived vaccine candidates conserved between human and mouse-adapted strains of *H. pylori*. Hum Vaccin 2008;4:219–223. [PubMed: 18376134]
18. Livingston BD, Newman M, Crimi C, McKinney D, Chesnut R, Sette A. Optimization of epitope processing enhances immunogenicity of multiepitope DNA vaccines. Vaccine 2001;19:4652–60. [PubMed: 11535313]
19. FDA Center for Biologics Evaluation and Research (CBER). Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Diseases Indications. Docket no. 96N-0400.

20. Gursel I, Gursel M, Ishii KJ, Klinman DM. Sterically stabilized cationic liposomes improve the uptake and immunostimulatory activity of CpG oligonucleotides. J Immunol 2001;167:3324–8. [PubMed: 11544321]
21. Rogers AB, Taylor NS, Whary MT, Stefanich ED, Wang TC, Fox JG. *Helicobacter pylori* but not high salt induces gastric intraepithelial neoplasia in B6129 mice. Cancer Res 2005;65:10709–15. [PubMed: 16322215]
22. Bamford KB, Fan X, Crowe SE, et al. Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. Gastroenterology 1998;114:482–492. [PubMed: 9496938]
23. D'Elios MM, Manghetti M, De Carli M, et al. T helper 1 effector cells specific for *Helicobacter pylori* in the gastric antrum of patients with peptic ulcer disease. J Immunol 1997;158:962–967. [PubMed: 8993017]
24. Tommaso, A Di; Xiang, Z.; Bugnoli, M., et al. *Helicobacter pylori*-specific CD4+ T-cell clones from peripheral blood and gastric biopsies. Infect Immun 1995;63:1102–1106. [PubMed: 7868233]
25. Lindholm C, Quiding-Jarbrink M, Lonroth H, et al. Local cytokine response in *Helicobacter pylori*-infected subjects. Infect Immun 1998;66:5964–5971. [PubMed: 9826379]
26. Haeberle HA, Kubin M, Bamford KB, et al. Differential stimulation of interleukin-12 (IL-12) and IL-10 by live and killed *Helicobacter pylori* in vitro and association of IL-12 production with gamma interferon-producing T cells in the human gastric mucosa. Infect Immun 1997;65:4229–35. [PubMed: 9317031]
27. Sommer F, Faller G, Konturek P, et al. Antrum- and corpus mucosa-infiltrating CD4+ lymphocytes in *Helicobacter pylori* gastritis display a Th1 phenotype. Infect Immun 1998;66:5543–5546. [PubMed: 9784570]
28. Lundgren A, Stromberg E, Sjoling A, Lindholm C, Enarsson K, Edebo A, Johnsson E, Suri-Payer E, Larsson P, Rudin A, Svennerholm AM, Lundin BS. Mucosal FOXP3-expressing CD4_CD25high regulatory T cells in *Helicobacter pylori*-infected patients. Infect Immun 2005;73:523–531. [PubMed: 15618192]
29. Raghavan S, Fredriksson M, Svennerholm AM, Holmgren J, Suri-Payer E. Absence of CD4+CD25+ regulatory T cells is associated with a loss of regulation leading to increased pathology in *Helicobacter pylori*-infected mice. Clin Exp Immunol 2003;132:393–400. [PubMed: 12780684]
30. Kaparakis M, Laurie KL, Wijburg O, Pedersen J, Pearse M, van Driel IR, Gleeson PA, Strugnell RA. CD4 CD25 regulatory T cells modulate the T-cell and antibody responses in *Helicobacter*-infected BALB/c mice. Infect Immun 2006;74:3519–3529. [PubMed: 16714584]
31. Lundgren A, Suri-Payer E, Enarsson K, Svennerholm AM, Lundin BS. *Helicobacter pylori*-specific CD4+ CD25high regulatory T cells suppress memory T-cell responses to *H pylori* in infected individuals. Infect Immun 2003;71:1755–1762. [PubMed: 12654789]
32. Thompson LJ, Danon SJ, Wilson JE, O'Rourke JL, Salama NR, Falkow S, Mitchell H, Lee A. Chronic *Helicobacter pylori* infection with Sydney strain 1 and a newly identified mouse-adapted strain (Sydney strain 2000) in C57BL/6 and BALB/c mice. Infect Immun 2004;72:4668–79. [PubMed: 15271928]
33. Garhart CA, Redline RW, Nedrud JG, Czinn SJ. Clearance of *Helicobacter pylori* Infection and Resolution of Postimmunization Gastritis in a Kinetic Study of Prophylactically Immunized Mice. Infect Immun 2002;70:3529–38. [PubMed: 12065492]
34. Ermak TH, Giannasca PJ, Nichols R, Myers GA, Nedrud J, Weltzin R, Lee CK, Kleanthous H, Monath TP. Immunization of mice with urease vaccine affords protection against Helicobacter pylori infection in the absence of antibodies and is mediated by MHC class II-restricted responses. J Exp Med 1998;188:2277–88. [PubMed: 9858514]
35. Huleatt JW, Cresswell J, Bottomly K, Crispe IN. $P27^{kip1}$ regulates the cell cycle arrest and survival of activated T lymphocytes in response to interleukin-2 withdrawal. Immunology 2003;108:493–501. [PubMed: 12667211]

36. Wolfraim LA, Letterio JJ. Cutting edge: p27Kip1 deficiency reduces the requirement for CD28-mediated costimulation in naive CD8+ but not CD4+ T lymphocytes. J Immunol 2005;174:2481–4. [PubMed: 15728451]

37. Shen R, Kaplan MH. The homeostasis but not the differentiation of T cells is regulated by p27(Kip1). J Immunol 2002;169:714–21. [PubMed: 12097373]

38. Díez-Juan A, Pérez P, Aracil M, Sancho D, Bernad A, Sánchez-Madrid F, Andrés V. Selective inactivation of p27(Kip1) in hematopoietic progenitor cells increases neointimal macrophage proliferation and accelerates atherosclerosis. Blood 2004;103:158–6. [PubMed: 14504088]

39. Rowell EA, Wang L, Hancock WW, Wells AD. The cyclin-dependent kinase inhibitor p27kip1 is required for transplantation tolerance induced by costimulatory blockade. J Immunol 2006;177:5169–76. [PubMed: 17015702]

40. Li L, Iwamoto Y, Berezovskaya A, Boussiotis VA. A pathway regulated by cell cycle inhibitor p27Kip1 and checkpoint inhibitor Smad3 is involved in the induction of T cell tolerance. Nat Immunol 2006;7:1157–65. [PubMed: 17013388]

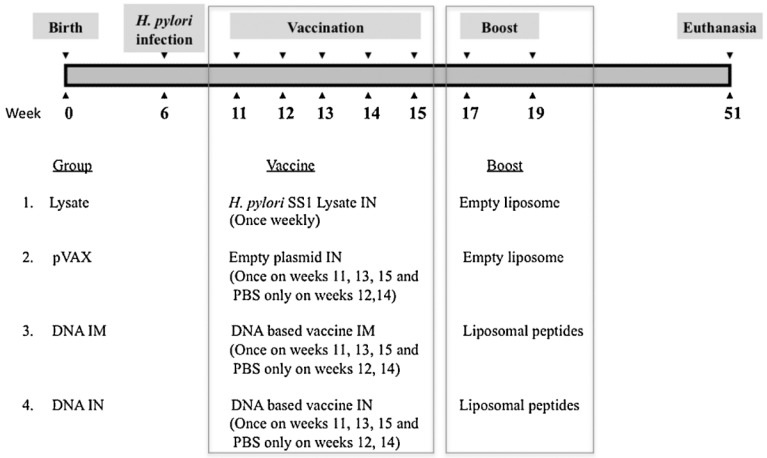

Figure 1.
Outline of study design.

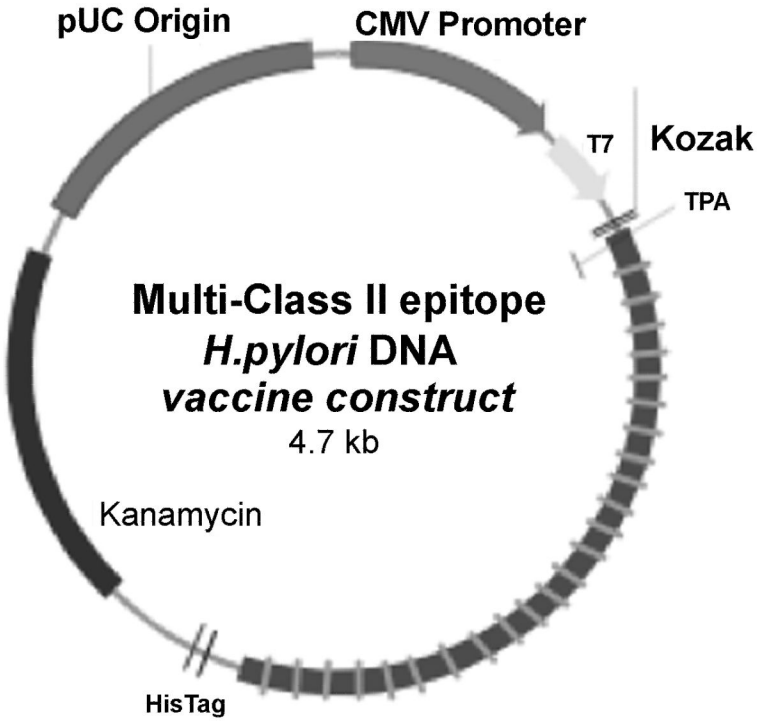

Figure 2.
Outline map of *H. pylori* DNA vaccine construct

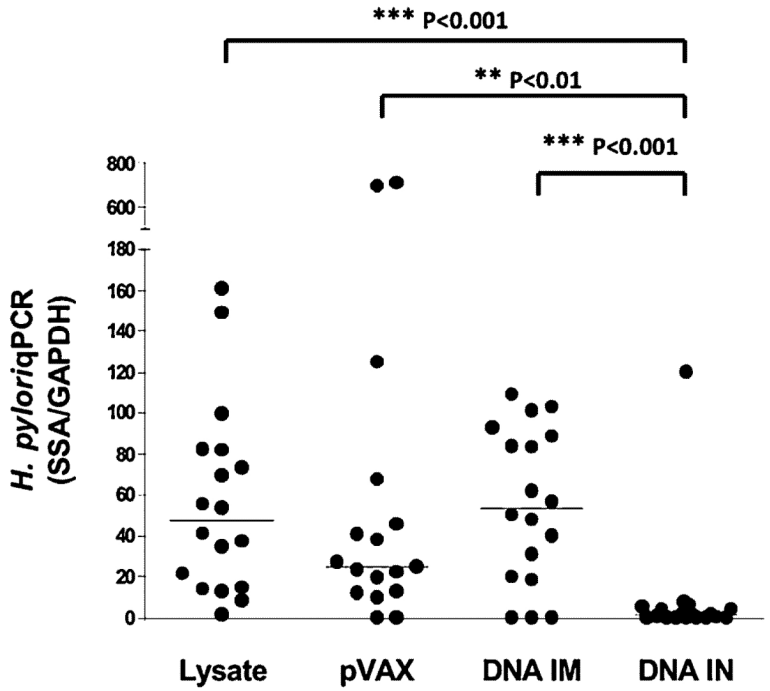

Figure 3.

H. pylori colonization. Quantification of gastric *H. pylori* colonization was performed by real-time PCR and expressed as the ratio of *H. pylori's SSA* gene to mouse GAPDH. Black bars represent median value of each group. Data analysis by Kruskal-Wallis oneway analysis of variance followed by Dunn's test demonstrate that the values for mice receiving DNA IN group were significantly lower than each of the other groups.

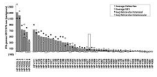

Figure 4.
Cell-mediated response to immunization of p27-deficient mice with HelicoVax A and HelicoVax B epitopes. Mice were primed with plasmid DNA vaccine and boosted with peptide pools comprising the HelicoVax A and HelicoVax B epitope sets or vaccine vehicle containing no epitopes. Splenocyte responses to individual and pooled epitopes were measured by IFN–γ ELISpot. Data are the mean spot forming cells (SFC) per million splenocytes for HelicoVax-vaccinated mice (filled bars), SS1 lysate-vaccinated mice (open bars) and HelicoVax DNA IN (filled circles) and DNA IM (open circles) groups of mice. Peptide pools 1 to 3 correspond to the epitopes contained in DNA vaccine construct A (HelicoVaxA), pools 4 to 6 to DNA vaccine construct B (HelicoVaxB).

Table 1

HelicoVax T-cell Epitopes. Column 1: peptide ID. Column 2: amino acid sequence. Column 3: GenBank accession number of parent protein. Column 4: sequence location in protein.

PeptideID	AA SEQUENCE	GenBank Accession Number	Location in Protein
HP 4001	NDNVRAYGGNIYEGVLINYYKAIDYMLLNDS	GI-15611087	139-173
HP 4007	RSKSKAKVRINDLRWVFSQRLSALVG	GI-15611162	73-99
HP 4009	MLLERSLIRVNKERLQTYLSLYANETSTRLSEV	GI-15611210	141-171
HP 4018	GLHFLGVFRFAFLYKTQSVGLASKS	GI-15611320	571-595
HP 4026	MHQYKRMQTYPVLFAIQKLALENN	GI-15611443	152-176
HP 4029	PAIYFANSSISGYVFLGLKTKRVRLDA	GI-15611469	42-69
HP 4032	AMGFNNYGAILGVRAFNRFAPYKTPIGINLGKN	GI-15611480	9-38
HP 4034	DSMIEIVNANFSLVHRYYHQKAQILGHK	GI-15611489	503-529
HP 4040	YDRWHGAFRLGYTYRINPFVGIYAQ	GI-15611518	165-192
HP 4042	LYFLGGVLRHARGLAAFTNASTNSYKRL	GI-15611528	22-49
HP4054	SDVVRTIEAYRMLRPLVIYPF	GI-15611636	340-371
HP 4055	TINVYYFNHGNLSFTYRRQYSLYVGYR	GI-15611681	67-101
HP 4060	LNTWLNMNALAPKILKALYAYGAQGINLGLNL	GI-15611745	177-208
HP 4061	FALVVFVLSVLLMFKQVRIWIYQYH	GI-15611753	174-201
HP 4064	HFSYKRYLINTLRKEFNFLGTPLILNA	GI-15611840	245-272
HP 4065	MKNFSPLYCLKKLKKRHLIALSLPLLSY	GI-15611844	14-42
HP 4067	CHALYNVGRISTYMLLGAITAGLGNS	GI-15611862	53-84
HP 4068	KIMYYILPQVWAWKKWRAKSLEKYCDF	GI-15611868	408-438
HP 4070	QESVKDAYSEFVFALKRLKILKAQMLELQKIN	GI-15611883	112-140
HP 4071	YNTYYKAGGAEVKYFRPYSVYWVYGY	GI-15611916	59-85
HP 4077	REEIEFMKRKYRLMWGKVADMSSVNK	GI-15612040	126-153
HP 4087	MPPIYRAENGLLVIRPLIKVREASS	GI-15612173	29-58
HP 4100	CKTMALALKALGVKRAMVVNGGGTD	GI-15612266	95-125
HP 4111	QKYFNDYLGLSSYGIIKYNYAQANNEKIQQL	GI-15612411	31-61
HP 4117	VWPFESLPSYLQVFVQIVPAYHGISLLG	GI-15612444	59-86
HP 4119	EIKVSSRQKNVALARKLVVYFARLYTPNPTLSLAQF	GI-15612482	49-78
HP 4120	GSSYHASLASVYLFERLAKIRARAILASEYR	GI-15612485	318-345
HP 4126	GEWYLVAMAYNYGLRKVQNAIKAAGTSD	GI-15612545	82-113
HP 4127	WKKLVKRFDVNYQFIPIIKNMLIGASVPQEFL	GI-15612545	47-77
HP 4152	DTAYKMLKANPTLALSAE	GI-15611119	723-740
HP 4153	DMLYKLNESLRIYQNVLSNNQDQL	GI-15612318	39-62
HP 4154	SNRFMLLWKNRYTLAKVQSFKLEPG	GI-15612384	78-102
HP 4156	FEKKLFVMNYPLTLYTTSPYGISEE	GI-15611791	56-80
HP 4157	MEQVIQNYRMIVALIQNKLSDA	GI-15611555	285-306
HP 4160	PFDLKLLEKEFYQNNATSLLVTS	GI-15611379	245-267
HP 4164	NMELKKALRHYLYAQTSNMVINCV	GI-15611135	173-196
HP 4165	GAFYKVWKNANAYIGTTGNPLGID	GI-15611410	344-367

PeptideID	AA SEQUENCE	GenBank Accession Number	Location in Protein
HP 4168	INSIPGYVKLFRKAYGSKVKID	GI-15612419	165-186
HP 4169	ESNISRRTAWGSWLQASAPYAIAPV	GI-15611318	115-139
HP 4171	PTNFVLGANIAGFRKVASAMIAQG	GI-15612066	424-447
HP 4174	LEDLLYLINRPAYANAKVSLQADF	GI-15612087	159-182
HP 4175	GAIITGNYALQAKLTGALFSEDK	GI-15612537	201-223
HP 4179	ELVALYQSAKALSAYWLEIE	GI-15611341	413-432
HP 4181	GLIYFFLNANTPSQHGF	GI-15612512	118-134
HP 4189	IKEYQYSGAFKAVFPLKVNQM	GI-15612027	73-93
HP 4192	TPGLLQFYRSALAYQLRDE	GI-15612041	297-315
HP 4193	FGQYKKLVNRFEGVLTGKGLTYGGS	GI-15612066	176-200
HP 4197	ASPYKNMLSLTLNAANGTISVSG	GI-15612049	405-427
HP 4198	LENYRSLNALFTRSLKKERPFDK	GI-15612340	46-68
HP 4199	ALKIEGRTKSSYYAAQTTRIYRLA	GI-15611225	250-273

Table 2

Gastric corpus histology was evaluated on a 0-4 scale [21] in mice euthanized 45 weeks after *H. pylori-infection*. Data are shown as median (range) of lesion index and analyzed by a Kruskal-Wallis one-way analysis of variance followed by Dunn's test.

Group	N	Median (range) of Lesion Index						
		Inflammation	Epithelial Defects	Oxyntic Atrophy	Hyperplasia	Pyloric Metaplasia	Dysplasia	Total
Hp Lysate	18	1 (0.5-2) *	1 (0.5-2)	0.5 (0-2.5)	0.25 (0-2)	0.5 (0-1.5)	0.5 (0-1.5) **	3.5(1-10.5)
pVax	17	0.5 (0-1.5)*	1 (0.5-2)	0 (0-1)	0 (0-1.5)	0 (0-2)	0 (0-1)**	1(0.5-8.5)
DNA IM	18	0.5 (0-3.5)	1 (0-2.5)	0.5 (0-1.5)	0.25 (0-3)	0.5 (0-2)	0.5 (0-2.5)	3 (0-15)
DNA IN	19	0.5 (0-1.5)	1 (0-1.5)	0 (0-1)	0 (0-1.5)	0.5 (0-1)	0 (0-1)	2 (0-7.5)

* Significantly more inflammation ($P < 0.05$), and dysplasia, comparing *H. pylori* lysate group with pVax control.

** Significantly more inflammation, and dysplasia ($P < 0.01$), comparing *H. pylori* lysate group with pVax control.