Special Section

The Development of Therapeutic and Preventive Vaccines for Gastric Cancer and Helicobacter pylori

Stephen Y. Chui, Timothy M. Clay, H. Kim Lyerly, and Michael A. Morse
Duke University Medical Center, Durham, North Carolina

Abstract

Gastric cancer is one of the most important worldwide public health problems. Convincing epidemiologic and etiologic associations have been made between the development of gastric cancer and infection with Helicobacter pylori. H. pylori not only has adapted to survive within the harsh environment of the stomach but also is able to modulate and avoid endogenous immune responses. The design and creation of efficacious vaccine strategies against H. pylori requires an understanding of the complex interactions that make up mucosal immunity. An effective vaccine strategy against H. pylori has the potential to affect significantly on population health worldwide. (Cancer Epidemiol Biomarkers Prev 2005; 14(8):1883–9)

Introduction

Human malignancies arise not only due to neoplastic transformation of cells but also due to tumor escape from host defense systems. Although currently still in the investigational stages of development, immunotherapy seeks to harness the body’s own surveillance and defense mechanisms to eliminate established disease and/or prevent future pathogenic processes. Unlike current interventions, such as cytotoxic chemotherapy and ionizing radiation, immune approaches to treat malignancy benefit from specificity: distinguishing the biological differences between normal and malignant cells to exert tumor cell death in a precise manner. In addition, advances in understanding the causes and processes of malignant transformation suggest the use of vaccine immune therapies to prevent the development of cancers. Greater understanding of both human immunoregulation and the basic biology of gastric cancer motivates inquiry and scientific development of immune-based therapeutic strategies.

Immune therapies against cancer exploit the unique and exquisitely sensitive innate mechanisms by which the body distinguishes and defends itself from foreign pathogens and malignant tumors. Antigenic targets that can be identified by the immune system as targets for destruction may either represent the unique products of mutated cancer-associated proteins or normal tissue antigens, which tumor cells overexpress or aberrantly express.

Therapeutic Vaccines against Gastric Adenocarcinoma

A clinically effective cancer vaccine is required to not only induce specific immune responses against unique tumor-associated proteins and peptides but also generate responses of sufficient magnitude and duration to cause tumor regression. Resultantly, novel vaccine strategies are designed to augment the apparently inadequate spontaneous antitumor immune responses in patients with gastric cancer.

Potential antigen targets of vaccine immunotherapy against gastric cancer include gastrin (a growth factor implicated in initiating signals for cell growth, proliferation, and metastasis) and the oncofetal protein carcinoembryonic antigen (CEA). Immune strategies targeting these antigens include the G17DT vaccine combining the gastrin protein with a highly immunogenic diphtheria toxin adjuvant (1) and also the fowlpox-CEA (6D)-TRICOM and vaccinia-CEA (6D)-TRICOM viral vector vaccines, which carry the gene for CEA in an immunogenic fowlpox or vaccinia viral expression vector along with a Triad of Immune Ostimulatory Molecules [[TRICOM-B7.1, ICAM-1 and LFA-3; ref. 2]]. Other therapeutic approaches for gastric cancer under development include an anti-idiotypic monoclonal antibody vaccine to CEA (3) intended to break tolerance to this self-antigen an induce both cellular and humoral immunity and vaccination with autologous tumor-derived heat shock protein conjugates (4). Similar to other studies of vaccination against solid tumors, these novel gastric cancer immune therapeutics show the capacity to increase immune responsiveness to antigenic targets. Yet, despite ongoing progress, induction of an adequate antitumor immune response to cause tumor regression remains an elusive goal.

Several obstacles lie in the way of creating a clinically efficacious vaccine immunotherapy for solid tumors, such as gastric cancer. The heterogeneity and genomic instability of neoplastic cells (5) may result in variable antigen production and expression (6) in turn limiting the effectiveness of immunotherapies that target a limited repertoire of tumor cell markers. Tumors may also avoid immune recognition by modulating expression of major histocompatibility molecules and thereby down-regulating antigen presentation (7). The fact that many gastric cancer-associated antigens (CEA, etc.) are self-proteins may promote the development of tolerance rather than immune reactivity to malignant cells. Finally, solid tumors may themselves be able to elicit a general suppressive effect on both immune effector cells and the immune system as a whole as evidenced by the lack of apparent reactivity of spontaneously occurring tumor-infiltrating lymphocytes (8). This environment of local immune exclusion may be created by cancer cells through mechanisms such as the potent Fas-Fas ligand signaling pathways (9, 10) and higher numbers of circulating regulatory T cells (11). Together, these mechanisms seem to safeguard a tumor from immune destruction while it spreads and grows.
The creation of an effective vaccine immunotherapy against an established gastric cancer will require the identification of the appropriate target antigens and a strategy capable of overcoming the varied and sophisticated immune escape mechanisms, which allow a tumor to grow. Because of the formidable challenges facing the development of gastric tumor immunotherapy, another area of promise for immunotherapy is in the prevention of gastric cancer.

**Helicobacter pylori** as a Causative Agent for Gastric Adenocarcinoma

*H. pylori* is a Gram-negative, spiral, microaerophilic bacterium that infects the human gastrointestinal tract and stomach. This human-specific bacillus is exquisitely adapted to survive within the hostile environment of the stomach. In most infected persons, *H. pylori* causes superficial chronic gastritis, which is clinically asymptomatic. However, in a significant proportion of people, *H. pylori* infection is also associated with the pathologic development of gastric and duodenal ulcers and gastroesophageal reflux disease with Barrett’s mucosa. Up to 1% of infected individuals will develop gastric adenocarcinoma, mucosa-associated lymphoid tissue lymphoma, or primary gastric non-Hodgkin’s lymphoma (12, 13).

Pathologically, *H. pylori*-associated gastric adenocarcinoma tends to be of the intestinal type. The histologic pathogenesis of *H. pylori*-induced intestinal type cancer may begin with chronic active gastritis and then proceed through the steps of atrophic gastritis, intestinal metaplasia, dysplasia, and finally cancer. Tumors of the body of the stomach tend to be associated with *H. pylori* more often than cardia tumors. Evidence of *H. pylori* infection is less frequently seen with diffuse-type gastric cancer (14). Molecular profiling studies have shown that intestinal and diffuse-type gastric cancers exhibit different patterns of gene expression (15), suggesting that these subtypes of malignancy evolve from different causative origins.

It has been estimated that half of the world’s population is infected with *H. pylori*, with higher rates occurring in persons belonging to lower socioeconomic strata. In developing countries, the prevalence of *H. pylori* infection increases rapidly throughout the first two decades of life until upwards of 80 to 90% of the population is infected by early adulthood. Up to 15% to 20% of infected individuals will eventually develop severe gastrointestinal diseases. With such an extensive distribution, *H. pylori* infection represents a significant and important worldwide public health problem causing up to 7 million cases of *H. pylori*-induced diseases occurring annually (16).

Geographic associations have been observed between rates of *H. pylori* infection and the incidence of gastric adenocarcinoma. At least nine retrospective case-control studies have looked at the association between serologic positivity for *H. pylori* infection and the incidence of gastric cancer. In *H. pylori*-infected individuals, the relative risk for developing gastric cancer was elevated from 1.2 to 4.2 (17, 18). The results of these cohort studies may be confounded by other factors potentially associated with gastric cancer that were not assessed, including dietary influences, and/or sampling exclusions based on prior histories of gastric symptoms or disease.

Several large-scale prospective population studies concluded that *H. pylori* infection is a risk factor for the development of gastric cancer. In a survey of 2,646 adults in a region with very high rates of gastric cancer in China, the presence of *H. pylori* at baseline was associated with a 1.8 odds ratio of increased risk of progression to dysplasia or gastric cancer (19). A prospective study of 1,526 Japanese patients with findings of duodenal ulcers, gastric ulcers, gastric hyperplasia, or non-ulcer dyspepsia on initial endoscopy reported that gastric cancer subsequently developed in 36 persons infected with *H. pylori*, whereas none of the uninfected persons developed gastric cancer (20).

These data implicate the presence of *H. pylori* infection influencing the development of gastric carcinogenesis. As the result, the WHO and IARC consensus group stated in 1994 that there was sufficient epidemiologic and histologic evidence to classify *H. pylori* as a definite carcinogen (21).

**Endogenous Immune Responses Do Not Clear H. pylori**

Very little is known about the natural history of *H. pylori* infection and the kinetics of spontaneously arising immune responses against the bacterium. Even the mode of person-to-person transmission of *H. pylori* remains unclear: oral-oral (22) and fecal-oral (23) routes of infection have been proposed. Once introduced into the gastric environment, *H. pylori* colonizes the mucus layer of the stomach lumen without attaching to host cells. Despite constant innate and immune responses against the pathogen, *H. pylori* manages to evade immune clearance, and in the absence of antibiotic therapy, infections persist for the lifetime of the host.

To survive the host’s immune defenses, *H. pylori* has evolved a multitude of unique characteristics and strategies. *H. pylori* shows extensive intrastrain and interstrain diversity, which assists the infecting agent evade immune recognition. Furthermore, the bacterium possesses the ability to downregulate strong proinflammatory responses and can also avoid and neutralize endogenous immune responses. Because innate immunity is inadequate to eliminate *H. pylori*, infectious complications may eventually develop, such as ulcerative disease, atrophic gastritis, intestinal metaplasia, mucosa-associated lymphoid tissue lymphoma, and/or gastric adenocarcinoma. It is unknown how bacterial genetic diversity, environmental factors, and/or host genetic background contribute to the development of pathogenic complications.

**The Rationale for Developing Vaccines against H. pylori**

Currently, treatment of symptomatic *H. pylori* patients consists of daily oral treatment with a proton pump inhibitor concurrent with at least two antibiotics, resulting in the eradication of infection in 80% to 90% of individuals (24). Although efficacious, pharmacologic treatment of *H. pylori* is complicated by patient compliance in taking a full course, high numbers of tablets, increasing resistance to commonly used antibiotics, and side effects, such as nausea and/or diarrhea (25). Importantly, the cost of multidrug therapy precludes the mass treatment of large populations of infected people, particularly in less developed countries where rates of *H. pylori* infection are much more prevalent. Additionally, reinfection remains an uncommon but important problem in regions where *H. pylori* colonization is endemic.

A vaccine that stimulated an efficacious immune response against *H. pylori* might be used (a) prophylactically to prevent infection and/or also (b) therapeutically to eliminate an established infection. Such approaches to treating *H. pylori* infection might reduce the incidence of late infection-related complications, including the development of gastric cancer, particularly when used as a population-based intervention in high prevalence areas of the world.

Is vaccination against *H. pylori* feasible? It has become apparent that inducing clinically effective immune responses against *H. pylori* will be problematic. The pathogenesis of *H. pylori* in humans is complex and multifactorial, with interplay between bacterial attributes and host response...
leading to chronic infection and persistent colonization. Furthermore, it is clearly difficult to achieve efficacious protective immune responses against *H. pylori* at mucosal surfaces given the insufficient nature of endogenous responses. Hampering the study of *H. pylori* infection is the fact that humans are the constitutive host: It is unclear whether animal models of infection with *H. pylori*, *Helicobacter felis*, or *Helicobacter mustelae* emulate human infection kinetics and chronicity. To illustrate the challenges facing *H. pylori* vaccine development, it is vital to understand how bacterial factors and host defenses interact in response to infection.

**H. pylori** Virulence and Antigenicity

*H. pylori* survives within the unique ecologic niche of the human stomach by colonizing the mucus layer that covers gastric epithelial cells. The bacteria buffers its own surface by converting gastric urea into ammonia and CO₂—the importance of this reaction is underscored by the presence of large amounts of urease produced by *H. pylori* (up to 5-10% of total protein content; ref. 26). Urease also seems to have an important function in promoting colonization of the gastric environment, because urease-negative *H. pylori* bacteria are unable to infect the stomach of gnotobiotic piglets (27). Despite the high level of this protein, it is unclear if urease represents a major immune response target—spontaneously arising urease-specific antibodies (28) and T cells (29) are found at low levels in *H. pylori*-infected individuals, even those with severe gastric pathology.

To promote survival in the human stomach, *H. pylori* also produces vacuolating cytotoxin A (VacA). VacA seems to insert into the cell membrane of gastric epithelial cells forming anion-selective channels, which promote osmotic swelling and fusion of late endosomes and lysosomes (30, 31). This induction of epithelial cell vacuolization alters the trafficking and endosomal processing of proteins. Importantly, VacA may influence the processing of peptides in antigen-presenting cells by down-regulating the generation of peptides capable of binding to MHC class II (32), although the in vivo effects of VacA on T-cell numbers and stimulation remain unclear (33, 34).

Studies of different *H. pylori* strains have shown an association between severe gastric pathologies (e.g., peptic ulcer and gastric cancer) and the expression of a bacterial gene encoding the cytotoxin-associated gene antigen (CagA). Conversely, *H. pylori* strains isolated from individuals with less severe gastric pathology (e.g., chronic gastritis) do not to express CagA and often also are devoid of VacA activity (35). CagA resides in a 40-kb pathogenicity island (PAI) of bacterial DNA that shares genetic and structural characteristics with PAI in other pathogens, such as enteropathogenic *Escherichia coli*, Bordetella pertussis, etc. Indeed, some of the genes in the *H. pylori* PAI seems to encode type IV secretory molecules that actively transfer molecules into eukaryotic cells (36). Through these mechanisms, CagA can actively translocate into the host cell cytosol and subsequently induce epithelial morphologic and functional changes (37). Endogenous immune responses to CagA are variable: During initial epithelial cell infection, CagA-specific humoral and cellular responses are high (38, 39). Later in infection, gastric biopsies from individually chronically infected with *H. pylori* show high levels of CagA-specific CD4⁺ T cells (40). It has been proposed that CagA or other PAI factors may be responsible for the skewing of immune responses toward the Th1 phenotype in *H. pylori*-infected individuals (41, 42) and experimentally infected animals (43, 44) perhaps by inducing the production of interleukin-8 and other chemokines by host cells. In addition to those mentioned above, numerous other bacterial virulence factors (such as neutrophil-activating protein) and structural/functional elements (flagellins, adhesions, heat shock proteins, etc.) may be targeted as antigens by novel vaccine therapies against *H. pylori* infection and disease. Some of these bacterial antigens are clearly recognized by the host immune system, although the host immunity is still not able to clear infection. Although the choice of antigen target(s) is clearly important, the development of an efficacious *H. pylori* vaccine will also depend on a broader understanding of the nature of mucosal immunity and the methods by which *H. pylori* evades host immune responses.

**How Do We Define Protective Immunity?**

A basic tenant of immunology is that prior immunity leads to protection against subsequent rechallenges by invading agents, such as bacteria and viruses. Vaccine strategies actively stimulate immune responses targeting specific antigens and/or pathogens. Prophylactic vaccination trains the immune system to recognize an antigen(s) as “foreign,” resulting in a subsequent vigorous adaptive response to future exposures, which prevents infection. Conversely, therapeutic vaccination activates the host immune system to clear an established infection. An efficacious therapeutic vaccine will elicit a preexisting memory response against an antigen and/or trigger an immune response against a target to which the host shows anergy or decreased responsiveness.

Protective immunity therefore requires that (a) the host recognizes an antigen as foreign and then (b) generates a defensive reaction intended to counteract the pathogen. However, the mere presence of an immune response does not always indicate that host protection is present. Importantly, a protective immune response needs to be of sufficient (c) magnitude and (d) duration to successfully clear the offending invader.

The design of a novel vaccine requires the careful selection of both appropriate antigen target(s) and an antigen delivery system that induces strong immune responsiveness rather than contributing to immunologic ignorance. For a pathogen, such as *H. pylori*, multiple virulence and pathogenic factors represent potential targets for active immunotherapy. Likewise, numerous vaccine adjuvants and technologies exist to deliver antigens for immune recognition and immune stimulation.

Advances in the field of systemic immunotherapy have been propelled by the development of analytic tools that allow quantification of immune responses to vaccine interventions. Powerful assays that measure immune biomarkers allow investigators to systematically ascertain the essential elements of systemic protective immunity, including measures of antibody specificity and titers (ELISA) and cellular immune specificity (peptide/MHC tetramer, ELISPot, etc.) and cellular immune activity (CTL function assay, etc.). Such immunologic assays allow investigators to study the size, quality, and durability of immune responses capable of protecting against infections, invaders, and tumors. These data gathered from the measurement of immune biomarkers have been used to optimize investigational immunotherapy interventions before formal clinical efficacy evaluation in human clinical trials. In similar fashion, tools capable of analyzing and quantifying the various key elements of mucosal immunity will be necessary to advance the development of vaccine strategies against persistent bacterial infections, such as *H. pylori*, at the mucosal interface between the body and the outside environment. Development of these assays will aid in discerning the functions of and interactions between the humoral and cellular immune compartments at the unique anatomic location of the mucosal surface. Quantification of mucosal immune responses will help to direct the rationale development of potent vaccines protecting against *H. pylori*. 
Mucosal Immunity and H. pylori

Mucosal immunity exists to provide protection against pathogens that infect and penetrate mucosal surfaces. The mucosal immune system encompasses a network of lymphoid structures, cellular immune elements, and antibodies work in concert to defend against entry through the gastrointestinal, urogenital, and respiratory surfaces. Because H. pylori lives in the lumen of the stomach, protection against this infection will depend on the optimization of effective mucosal immunity.

The principal antibody involved in mucosal immune protection is secretory IgA—a complex of two antibody molecules that post-translationally associate together by binding a J chain. The J chain functions not only to hold the two IgA molecules together but also to promote endosome transport through epithelial cells and additionally facilitate binding to the poly-Ig receptor on the luminal side of the epithelial cells (45). The presence of secretory IgA prevents adhesion of viruses, bacteria, and toxins to luminal surfaces, thereby preventing pathogens from crossing the epithelial barrier. Up to 40 mg/kg body weight of IgA are secreted daily by humans, underscoring the importance of protective immunity at mucosal surfaces and the important role of secretory IgA (46).

Animal experiments suggest that IgA is the most important effector molecule involved in mucosal immunity and the defense against bacteria that inhabit the lumen of the stomach, such as H. pylori. Protection against H. pylori and H. felis infection in several mouse models seems to correlate with the increasing levels of mucosal IgA (47, 48). Furthermore, administration of anti-Helicobacter IgA monoclonal antibodies seems to protect animals from subsequent challenge with H. felis (49). There is also some evidence that IgG antibodies may also contribute to immunity against H. pylori (50).

However, despite the important role of antibodies in mucosal immunity, it seems that antibody-dependent mechanisms alone are not sufficient to defend against infection with bacteria, such as H. pylori. For instance, the stimulation of high levels of mucosal IgA using a novel intranasal vaccine in mice does not necessarily indicate protection against H. pylori or H. felis infection (51). Although immunoglobulins, and in particular IgA, play a very important role in protective immunity at mucosal surfaces, it is apparent other immune mechanisms are necessary to successfully defend against infecting organism.

Cellular immune effector and regulatory cells exhibit responsiveness to infection of gastric mucosal surfaces by pathogens and parasites, such as H. pylori. Mucosa-associated T cells (also known as intraepithelial lymphocytes) home to Peyer’s patches, the lamina propria, and the intraepithelial compartment. In animal models of H. felis infection, both CD4+ and CD8+ populations of T cells are seen to progressively infiltrate into gastric tissues, with the degree of gastric leukocyte infiltration seeming to correlate with protection against H. pylori in murine models (52). Immunization of mice incapable of antibody production has been shown to prevent infection by H. pylori, implying an important role for cellular immune effectors, such as T cells, in mucosal protective immunity (53, 54). In humans, higher H. pylori density in the stomach is characterized by increased gastritis, indicating the presence of a vigorous gastric inflammatory response to infection (55). Unlike animal models, however, it is unclear whether a strong human gastric inflammatory response to H. pylori is protective as illustrated by the association between H. pylori infection and chronic gastritis (56).

The phenotype, kinetics, and most importantly the significance of the cellular immune response to H. pylori infection remain perplexing. The recruitment of Helicobacter-specific T cells into gastric tissues confirms the reactivity of T-cell adaptive immune lymphocytes to this infectious bacteria (56).

H. pylori Evasion of Host Immune Responses

H. pylori has evolved numerous mechanisms to avoid and subvert immune destruction. H. pylori may attenuate innate immune responses by producing arginase, which inhibits nitric oxide production by activated macrophages (63). Additionally, bacterial uptake by phagocytic cells may be actively blocked by H. pylori expressing a type IV secretion system encoded within the CagA PAI (64, 65). H. pylori has also evolved mechanisms to undermine adaptive immune responses. The 95-kDa secreted protein VacA not only induces cellular vacuolization in epithelia cells but also seems to interfere with interleukin-2 signaling pathways in T cells by blocking intracellular calcium mobilization and the subsequent activity of the calcium-dependent phosphatase calcineurin (66, 67). VacA also interferes with MHC class II–mediated antigen presentation by altering the endocytic environment in which protein processing occurs (68).

H. pylori is also capable of modulating host inflammatory reactions and thereby circumvents an important mechanism by which the host eliminates infective pathogens. It has been observed that a vigorous gastric inflammatory reaction to H. pylori infection may lead to reduction or even clearance of bacteria: The increased inflammatory responses in interleukin-10 knockout mice results in elimination of H. pylori infection (69) and the development of atrophic gastritis in H. pylori–exposed individuals is associated with the loss of overt bacterial infection (70). However, H. pylori has evolved several characteristics, which intrinsically decrease the likelihood of provoking inflammatory reactions. Although bacterial lipopolysaccharide is a primary mediator of phagocyte, lymphocyte, and endothelial and epithelial cell activation, H. pylori lipopolysaccharide has very low proinflammatory activity. Compared with E. coli lipopolysaccharide, H. pylori lipopolysaccharide has 2,000–3,000 fold decreased ability to activate macrophages (71). In addition, H. pylori flagellins have markedly reduced capacity to activate TLR5 (a member of a family of phylogenetically conserved mediators of innate immunity) compared with flagellins from other Gram-negative bacteria (72).

Genetic diversity and adaptability also permits H. pylori to survive within the hostile and changing gastric mucosal
An effective vaccine has the potential to prevent late problems arising from \textit{H. pylori} infection, such as gastritis, peptic ulcer disease, and even gastric malignancies. Multiple methods and novel strategies have been used to determine potential antigen targets and overcome the varied mechanisms, which allow \textit{H. pylori} to escape immune surveillance and immune destruction.

Potential antigen targets for vaccine immunotherapy include the urease enzyme as well as the virulence determinants, including the cytotoxic VacA protein, the CagA pathogenicity factor and other PAI elements, heat shock proteins, and chemotaxis proteins, such as CheY1, CheY2, etc. (76). The choice of antigen delivery system and the route of immunization can also affect both the magnitude and the quality (Th1 versus Th2, etc.) of an immune response. Numerous \textit{H. pylori} immunization strategies are currently under development, including vaccines incorporating immunostimulatory adjuvants, such as cholera toxin and/or \textit{E. coli} heat-labile toxin (77, 78), live attenuated vectors (79, 80), DNA immunization (81), encapsulated nanoparticles (82), etc. Mucosal (oral) and nonmucosal antigen delivery has also been studied in an effort to identify the most efficacious route of immunization (83).

Although there are numerous antigenic targets and technologies to deliver them, perhaps the most critical facet for optimizing the development of \textit{H. pylori} vaccines is a greater knowledge of the mechanisms by which a mucosal immune response is able to protect and/or eliminate a pathogen. Although studies continue to better delineate the components and the complex dynamic mechanisms that make up the mucosal immune system, we still lack a definition of what immune effectors/immune interactions make up protective immunity. At its worst, vaccine development proceeds along a haphazard way in the hopes of “stumbling” on a clinically effective agent. On the other hand, the study of immunostimulatory vaccines and mucosal immune analysis can also complement each other and add to the understanding of both immune modulation and mucosal immune structure. Tools capable of easily and serially assaying mucosal immune effector elements are necessary to evaluate the effects of potential stimulatory vaccines. A greater understanding of the mechanisms of protective mucosal immunity will help to guide the development of vaccines against \textit{H. pylori}.

**References**


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