Special Section

Vaccination as a Method of Preventing *Helicobacter pylori*–Associated Gastric Cancer

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Introduction

Helicobacter pylori is a Gram-negative, microaerophilic, spiral bacterium (1), which was first discovered >20 years ago in Perth, Australia (2) and continues to infect >50% of the world's population (1). The prevalence of infection ranges from 20% in the developed/industrialized countries to >90% in the developing world (3). The organism itself is highly motile and resides in the mucus lining of the human stomach, where it thrives in part by neutralizing the stomach acidity by production of urease (3). *H.pylori* has been firmly established to be responsible for chronic gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma (4). WHO has classified *H. pylori* as a class I human carcinogen and the NIH recommended treating *H. pylori* for cure of ulcers (ref. 3, Yamada, 1994 #16). The Maastricht 2-2000 consensus report not only recommended eradication of *H. pylori* in all patients with peptic ulcer but also in *H. pylori*–infected individuals with low-grade gastric mucosa-associated lymphoid tissue lymphoma, atrophic gastritis, following gastric cancer resection and first-degree relatives of gastric cancer patients (5). Eradication of *H. pylori* is a daunting task, which is met with major challenges (6). Currently, acid suppression with a proton pump inhibitor and two (triple therapy) antibiotics taken together has been the treatment of choice (5). The emerging bacterial resistance may necessitate the use of quadruple therapy with three antibiotics (5). In addition, these antimicrobial agents must penetrate the mucous barrier, be active at acidic pH, and must remain in contact with the surface long enough to be effective (6). The complexity of this eradication therapy however results in poor patient compliance, and the cost of these drugs is prohibitive in nations where *H. pylori* is endemic (7). Finally, from an immunologic perspective, even successful eradication therapy does not protect the host from potential reinfection nor prevent asymptomatic infected individuals from developing gastric cancer. Therefore, there would be tremendous benefit to the society if safe, effective, and cost-effective vaccines were available to prevent or cure chronic *H. pylori* infection (7). There are major obstacles in the therapy of *H. pylori*. Currently, favored ''search and eradicate'' strategy (8) is being replaced in favor of ''diagnoses, treat, and confirm cure'' approach (6).

Host Immune Response

H. pylori infection induces a histologic gastritis, even in asymptomatic individuals with both acute and chronic characteristics (3). Chronic infection induces both local and systemic adaptive immune responses, but this response is ineffective in clearing the bacteria.

Because *H. pylori* is a mucosa-associated organism, it was initially thought that an IgA type anti-*Helicobacter* antibody response would be essential for protective immunity (2). Subsequent studies provided compelling evidence that a humoral response was not required for protective immunity against gastric helicobacters (2). When μMT mice, which are deficient in B-type lymphocytes, were prophylactically immunized and then challenged with *H. pylori* or *Helicobacter felis*, excellent protection was achieved showing that antibody-independent immunity is possible against *H. pylori* (2).

In response to *H. pylori* infection, neutrophil trafficking is noted in the gastric epithelium and gastric glands (3), whereas lymphocytes, which are usually absent from the stomach, are found in abundant numbers throughout the lamina propria (3). *In vitro* studies have shown that the peripheral blood mononuclear cells and the lamina propria–derived mononuclear cells from *H. pylori*–infected individuals respond to stimulation by *H. pylori* antigens with the production of cytokines such as IFN-γ (3). This observation suggests that *H. pylori* induces a Th1-mediated proinflammatory response that recruits $CD4^+$ T cells resulting in the increased local production of cytokines such as IFN-γ and interleukin-12 (IL-12; ref. 2). It should noted, however, that the TH1 response alone is not enough to cure this infection (2).

Vaccine Prototypes

It has always been a challenge to decide upon the most efficacious method of vaccine delivery. It was initially argued that oral vaccination would probably be the best route because *H. pylori* is a noninvasive pathogen and effective mucosal immunity would be the key to eradication. Because proteins can rapidly degrade when exposed to the acidic content in the stomach, it was problematic to find the right vaccine that could survive this environment and still retain its efficacy. Another problem which continues to be a challenge for the development of oral vaccines is the availability of a suitable adjuvant (2). An adjuvant is an important component of any oral/mucosal vaccine as it is responsible for stimulating immune system, but due to toxicities associated with these agents, there are currently no suitable and safe adjuvants available for use in humans. Another important limitation to effective oral immunization is that it requires multiple doses and a large amount of antigen administration.

Oral Immunization. Cholera toxin has 5 B subunits that bind to cellular GM1 ganglioside and a single A subunit with ADP ribosylation activity. Cholera toxin binds to Peyer's patches and at low doses has been found to be a potent mucosal adjuvant. It has also been noted that it is an effective adjuvant without the need for covalent conjugation to the antigenic proteins. Cholera

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toxin also acts to increase the vascular permeability, which may lead to increased antigenic uptake and may facilitate isotype switching to IgA. Similarly, a related mucosal adjuvant, *Escherichia coli* LT has also generated a lot of interest but was found to cause cramping and diarrhea (9).

Alternative Routes to Mucosal Immunization. Considering the side effects of oral adjuvants, researches have focused attention on making oral immunization safe and effective for human usage. These efforts have been fraught with multiple problems including safety. As stated above, *E. coli LT* that was used as an oral adjuvant in humans did show a significant decrease in gastric *H. pylori* density but was associated with cramping and diarrhea (9). Different strategies are being tested to make oral immunization safe as well as effective in humans as one approach that seems to be gaining momentum is to administer *H. pylori* antigens with nontoxic LT mutants (10), which are produced by inducing point mutations. Another approach is to use other mucosal routes such as the nasal mucosa and the rectal mucosa for effective immunization. When mice were immunized orally, rectally and intranasally in combination with *E. coli* heat-labile toxin (LT) and subsequently challenged with purified *H. pylori* antigen, mice immunized by either of the routes were found to be protected against this challenge compared with controls (11). These studies showed that mucosal immunity can be induced by oral or intranasal routes of immunization. The intranasal route of immunization is similar to the oral route in that it also requires the administration of bacterial antigen in conjunction with an adjuvant multiple times (3). However, significantly less antigen and adjuvant is required as compared with the oral route (11).

Systemic Immunization against *H. pylori* Infection. The search for an effective immunization route is ongoing. Although the intransal route of immunization seems the most efficient and effective route of mucosal immunization, it still has some major shortcomings. Administration of a mucosal adjuvant is still necessary and some studies have shown that CT and LT can induce histologic inflammation in the olfactory bulb and cause paralysis of facial nerves (ref. 3, Fujihashi, 2002 #34). Intranasal route could also result in oral ingestion thus exposing the subject to various side effects and toxicities. In a recent study conducted by Eriksson et al. (12), CTA1-DD adjuvant was found to be safe for intranasal administration without any accumulation in the nervous tissue. Thus, researchers are aggressively pursuing intranasal immunizations with both CT and CTA1-DD.

Other modalities of administering vaccines such as the i.p. and s.c. routes are also being pursued. Mice were vaccinated i.p. using *H. pylori* antigen in combination with aluminum hydroxide and upon rechallenge with *H. pylori* were found to confer protection that was shown by absence of bacteria, both histologically and in culture of gastric biopsy tissues (13). This immunity was noted to be antibody independent (13) and achieved by IL-5-secreting T cells. Finally, it was possible to transfer protective immunity from immunized mice via $CD4^+$ T cells to $rag1^{-}/^{-}$ mice thus proving that antibody production was not necessary for effective immunity (13).

In a similar experiment done on neonatal mice, which were immunized by a single dose of the vaccine consisting of complete Freund's adjuvant and *H. pylori* lysate, Eisenberg et al. showed effective immunity via production of antigen-specific IFN-γ, IL-2-, IL-4-, and IL-5-secreting T cells (14). The results of these experiments suggest that until a safe, effective, and inexpensive mucosal (oral/nasal) vaccine becomes available, systemic immunizations should be a consideration.

Clinical Trials

Based on studies in mice, pilot studies in humans have been conducted using oral vaccines containing either 180, 60, or 20 mg of urease with 5 μg of LT given in four weekly oral doses. Sixty-three percent of the volunteers in the study, reported abdominal cramping and diarrhea within 12 to 24 hours after administration of the vaccine. A significant improvement was noted when the investigators decreased the dose from 10 to 5 μg (9). On a positive note, this vaccine resulted in a significant increase in IgA-anti-urease antibody ($P = 0.018$) and a decrease in the *H. pylori* bacterial load. Several investigators are currently involved in trying to find a vaccine formulation, which would confer sterilizing immunity as well as be free of the debilitating side effects of the previous formulations. This has generated a lot of interest among researchers and will hopefully aid in the development of an effective vaccine in the future.

Conclusion

The only modality available for *H. pylori* eradication today is by the use of multidrug cocktails that have the disadvantages of compliance and organism resistance. Because eradication regimens have various shortcomings, the only real effective way of dealing with *H. pylori* is prevention by way of immunization. Great strides have been made in mouse animal models after administration of oral vaccines, which holds promise for the future. Mucosal immunization will hopefully be helpful in generating herd immunity thus protecting unimmunized individuals as well. However, despite the fact that *H. pylori* is a mucosal pathogen, protective immunity can be achieved by mucosal as well as parenteral administration of vaccines. This suggests that successful sterilizing immunity against *H. pylori* and the prevention of gastric cancer can be safely achieved now using conventional systemic vaccines designed to optimize immune responsiveness (3).

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