Vaccine development against infection with *Helicobacter pylori*

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Infection with *Helicobacter pylori*, is one of the most prevalent infections worldwide, where approximately 50% of adults in the developed world and over 90% of inhabitants in the developing world are infected. Chronic infection with *H. pylori* is the cause of gastritis, peptic ulcer disease and is a risk factor for gastric adenocarcinoma. Recent studies have demonstrated the suitability of an immunization strategy in the prevention and treatment of *H. pylori* infection, and the potential for management of disease. Mucosal administration of purified recombinant sub-unit proteins of *H. pylori*, together with a mucosal adjuvant, has identified urease to be highly efficacious in prophylactic and therapeutic animal model studies, and show partial therapeutic activity in humans. Several other antigens are also effective, and the recent sequencing of the *H. pylori* genome has led to an intensive effort in antigen discovery. Other research has centered on the identification of novel approaches for delivery, and the immunological mechanisms underlying protective immunity. In this review, preclinical data and the results of early-stage clinical trials and directions for future research on *Helicobacter* vaccines are described.

Development of a vaccine against *H. pylori* infection

Rationale for vaccine development

Immunization strategies are an effective and economical approach to the prevention and control of infectious disease. The role for an immunization approach in the prevention of chronic diseases, like peptic ulcers and gastric cancer, is an exciting new area of development. Although many antimicrobial treatment regimens against *H. pylori* infection are effective in patients with active duodenal ulcer disease, the ability to treat does not obviate the need for a preventive strategy. In fact, most *H. pylori* infections leading to peptic ulcer disease and gastric cancer occur in individuals who have sustained long-term infections without symptoms.

The application of vaccines for treatment of infectious diseases although in its infancy, if successful, will have a tremendous impact not only on the management of chronic infections such as *H. pylori*, but also HIV, viral
Helicobacter infection

Helicobacter infection, chlamydia, and a wide range of parasitic infections. The underlying concept being that the vaccine could redirect or alter the host immune response in such a way that the pathogen's ability to evade immunity is diminished and clearance of the infection is achieved.

In the developed world, the effectiveness of conventional treatment regimens has diminished interest in therapeutic vaccines. However, vaccines used in combination with antibiotics, could improve the rate of treatment success, decrease the rate of antimicrobial resistance and prevent reinfection and disease recurrence. Clearly, natural immunity to H. pylori is ineffective in preventing reinfection, as has been demonstrated in animals and limited studies of humans who have been followed after successful eradication of infection. In areas of the world where reinfection rates are high after antimicrobial treatment, concurrent prophylactic immunization will be an essential component to therapy. In the developed world, reinfection rates in adults appear to be low (0.5–2%) and, therefore, do not pose a problem for antimicrobial therapy in disease recurrence. However, reinfection rates may be higher in children, where, in one study, 18% of children became re-infected within 18 months of antibiotic therapy.

Since H. pylori infection is acquired principally in childhood and is associated with a high risk of disease decades later in life, it is reasonable to consider preventative interventions prior to disease onset. A wide array of simple, office-based serological screening tests are now available for identifying infected individuals, and can now discriminate infection with the more virulent H. pylori phenotype, notably CagA. These methods could be used to identify subjects with H. pylori gastritis during the first two decades of life, who are at future risk of ulcer disease and cancer. If treatment of the infection is considered, co-administration of a vaccine to prevent reinfection may be an important component of such an intervention strategy. Childhood immunization to prevent chronic disease acquired decades later is not without precedent, and underlies the recommendation for universal immunization against hepatitis B, a disease that causes considerably less cancer morbidity and mortality than H. pylori.

Experimental evidence for the feasibility of an immunization strategy

Consideration of vaccination as a means to control peptic ulcer disease began around 1990. Pallen and Clayton suggested that urease would be a candidate antigen for incorporation in an H. pylori vaccine. Czinn and Nedrud showed that H. pylori whole cell sonicates administered intragastrically to mice and ferrets elicited serum and intestinal IgG and IgA antibodies. Subsequent studies demonstrated that mice orally immunized with Helicobacter sonicates or whole cells and cholera toxin (CT) adjuvant were protected against challenge with H. felis. In addition, passive protection against challenge by the oral administration
of an IgA monoclonal antibody, later shown to be specific for Helicobacter urease\textsuperscript{10}, was demonstrated, suggesting that the principal mediator of protection after active immunization may be secretory IgA. In 1994, Michetti \textit{et al}\textsuperscript{11} demonstrated that mice orally immunized with recombinant \textit{H. pylori} urease were protected against challenge with \textit{H. felis}. A large body of data has now accumulated from several laboratories confirming that urease administered mucosally confers protection against challenge\textsuperscript{12-14}. While initial immunization studies utilized \textit{H. felis} as the challenge, development of an \textit{H. pylori} model confirmed that urease protected against the human pathogen itself\textsuperscript{15-17}. The role of mucosal immunity in protection against \textit{H. pylori} in humans is also supported by a study of infants in West Africa, where infection occurs within the first year of life. Infants of mothers with high titers of anti-\textit{Helicobacter} IgA in breast milk had a significant delay in acquisition of \textit{H. pylori} infections\textsuperscript{18}. Subsequent studies indicate that the principal antigen recognized by breast milk IgA is urease (Thomas J., personal communication, 1996).

In 1994, Doidge \textit{et al}\textsuperscript{19} reported that mice with sub-chronic \textit{H. felis} infections either cleared or had reduced infection after oral immunization with \textit{H. felis} whole-cell sonicates. Urease administered orally to mice experimentally infected with \textit{H. felis}\textsuperscript{20} or ferrets naturally infected with \textit{H. mustelae}\textsuperscript{21} was also shown to have significant therapeutic activity. These studies indicated that the up-regulation of immunity to specific \textit{H. pylori} antigens may result in clearance of chronic infection, and stimulated efforts to investigate this possibility in humans.

**Approaches to development of a vaccine for humans**

Although the feasibility of an immunization approach has been established, the development of a safe and effective vaccine for human use remains an active area of research. The use of whole bacteria or cell extracts is potentially problematic and, while recombinant sub-unit vaccines, like urease, are attractive alternatives, additional antigens may need to be included. Additionally, the selection of an effective method for presentation of antigen to the host’s immune system, ensuring the induction and recruitment of a protective immune response is critical.

**Prophylaxis in a model of Helicobacter infection**

**Murine efficacy studies**

Studies in a murine model of \textit{Helicobacter} gastritis have been performed to determine the optimum route and schedule of vaccination, the dose-activity relationships, the requirement for adjuvants, and the immunological
correlates of activity. Mice given rUrease by the oral route at doses as low as 50 ng were significantly protected against intragastric challenge with $>10^3$ 90% infectious doses of virulent H. felis. A comparison of several immunization schedules demonstrated optimal protection and immunogenicity when 4 doses of $\geq 1 \mu g$ were given at intervals of 7 days. A mucosal adjuvant, such as CT or LT, was required for the induction of protective immunity. Administration of the antigen alone did not protect by any route, even when given in large doses resulting in the stimulation of high levels of urease-specific serum IgG and salivary IgA antibody. Duration of protection induced by rUrease was determined by challenging groups of mice at intervals up to 10 months after immunization. During this period of observation, IgG and IgA antibody levels in serum and saliva persisted, with no decrease in resistance to challenge. In a similar study, groups that were immunized and challenged and then followed over the course of 13 months remained solidly protected, demonstrating that the infection did not recrudesce over time.

Protection against H. felis in these and previous studies was measured by decreased urease activity in gastric tissue and by examination of stained gastric tissue for bacteria. The former method is relatively insensitive, the limit of detection being approximately 1,000–5,000 bacteria. To determine more accurately the level of protection afforded by vaccines and to measure protection against the human pathogen, we developed a murine model of H. pylori infection, and utilized quantitative culture as a highly sensitive read-out. Using this model, a large number of prophylactic and therapeutic experiments have been completed. In one such study, mice received 25 $\mu g$ rUrease by the oral or rectal routes or 10 $\mu g$ by the intranasal (IN) route, together with LT adjuvant. Animals were challenged with $10^3$ ID$_{90}$ of H. pylori 2 weeks after completion of the fourth weekly vaccination, and were sacrificed 2 weeks after challenge to assess residual gastric infection by quantitative culture. Significant protection was observed in all vaccine groups ($P < 0.05$). Importantly, immunization did not prevent colonization completely, but markedly reduced the mean bacterial density by 100- to 1000-fold. Similar results have been reproduced many times, and indicate that complete protection (‘sterilizing immunity’) is achieved in only 10–20% of animals, whereas the remaining animals show a reduced bacterial burden. Because of the sensitivity and reproducibility of the model, it provides a means of investigating other modalities for improving the efficiency of immunization.

Although the marked decrease in bacterial density observed in immunized animals is encouraging, the objective of achieving complete protection remains important for several reasons. The most important of these is the practical difficulty of designing clinical trials in which the
outcome measurement is reduced bacterial density or reduced severity of disease rather than absence of infection. Although, gastric bacterial density appears to correlate with pathology\textsuperscript{24}, protection against a high level infection may be sufficient to delay or even prevent the onset of disease. For these reasons, preclinical studies are currently focused on achieving full protection in mice and other hosts. The residual infection in our model may be due to one of the following: (i) the high challenge inoculum (1000 ID\textsubscript{50}), resulted in a breakthrough of the level of immunity achieved by vaccination; (ii) urease shed from the bacterial surface may act as a decoy for antibody; (iii) residual \textit{H. pylori} may occupy an immunologically privileged site that is inaccessible to antibody or the effector function of T-cells; (iv) the need for alternate antigens and/or adjuvants to stimulate a more effective response; or (v) reduced levels of urease on the surface of \textit{H. pylori} cells as the bacterial density decreases.

To address some of these issues, we initiated investigations of sub-unit antigens other than urease. At the present time, 8 novel \textit{H. pylori} antigens other than urease have shown significant prophylactic activity in the mouse model, including both rHspA (a GroES homologue) and catalase. Protection of mice with HspA and catalase has also been demonstrated by Ferrero \textit{et al}\textsuperscript{25} and Kolesnikow \textit{et al}\textsuperscript{26}, respectively. Ongoing studies in our laboratory include the co-administration of HspA, catalase, and several other protective antigens by simultaneous or sequential mucosal administration together with urease.

Additional experiments with different \textit{H. pylori} isolates, revealed that challenge with the Sydney (SSI) strain resulted in a chronic infection that was two-logs below that observed for our challenge strain. When SS1 was used as a challenge inoculum in an efficacy study, immunization with rUrease resulted in an equivalent 2–3 log reduction in bacterial burden as routinely observed, although in this instance ‘sterilizing immunity’ was achieved (unpublished data). The difference in the level of infection/efficacy possibly reflects differences in host adaptation, and possible phenotypic differences expressed by these \textit{H. pylori} strains. This may suggest that ‘sterilizing immunity’ is strain dependent, or, that protective immunity employing mucosal immunization against infection with our current challenge strain (more analogous to the level of infection observed in human biopsy material; \(\sim 10^5\)/tissue sample), results in a persistent but reduced infection.

The criteria for inclusion of an antigen in a final vaccine formulation include: (i) efficacy when used as a single component and additive or synergistic effects when combined with urease; (ii) compatibility with scale-up production and purification; (iii) lack of toxicity and cross-reactivity with human tissue antigens; and (iv) conservation among \textit{H. pylori} isolates from multiple geographic locations. The latter criterion
may reduce the utility of antigens such as CagA or VacA which are expressed by only a subset of natural isolates or LPS, which is antigenically variable and contains cross-reactive Lewis blood group antigens^{27}.

The role of immune responses in protection

Because it is an intraluminal infection, immunity to \textit{H. pylori} is probably mediated, at least in part, by secretory IgA (slgA) antibodies. A role for slgA in protection can be inferred from studies of passive immunity. Human breast milk IgA titers correlate with protection of infants against early acquisition of \textit{H. pylori} infection^{18}. Orogastric administration of a monoclonal IgA antibody directed against urease passively protected mice against \textit{H. felis} challenge^{9,10}. These observations demonstrate that colonization by \textit{Helicobacter} spp. is preventable in the presence of adequate levels of slgA and support the selection of urease as a vaccine candidate.

The immunological basis for protection observed in mice after active immunization remains uncertain. In general, protection appears to correspond with the development of IgA antibody responses in gastric secretions and with the recruitment of T and B cells into the gastric mucosa. The immunological specificity and function of the T cells found in gastric tissue of vaccinated mice have not been defined. However, they appear to be of intestinal origin, indicating that their presence is a result of mucosal immunization. The antigen-specificity of B cells in the gastric mucosae of immunized-challenged mice has been determined by immunohistochemistry; these cells are predominantly IgA+ and 10–20% of these cells contain antibody directed against urease (unpublished results). Gastric mucus sampled with cellulose wicks contains both IgA and IgG antibodies against urease. The IgG subclass ratio of these samples is identical to that found in serum, and we have found no evidence for IgG antibody-containing cells in gastric mucosal tissue of immunized mice^{17}. Our results indicate that IgG in gastric mucus is the result of transudation from serum rather than local production, and that serum IgG alone is not protective.

Employing mucosal immunization with rUrease and LT raises antibodies in serum, saliva and feces, but does not elicit a gastric immune response prior to challenge with \textit{H. pylori}. However, when immunized mice are challenged the stomach is transiently colonized by \textit{Helicobacter}, resulting in immunologically specific and nonspecific T and B cells being recruited to the gastric mucosa. IgA antibody containing cells are absent from the gastric mucosa of immunized mice, whereas upon challenge with \textit{H. pylori} large numbers of such cells are recruited, significantly higher than observed in unimmunized controls.
Similarly, a gastric T cell response occurs in immunized mice after challenge with *H. felis* or *H. pylori* \(^{17,28}\), whereas no response is seen in unchallenged, immunized mice. In a study reported by Mohammedi *et al.*, adoptive transfer of a Th2 cell line, but not Th1 cells resulted in clearance of bacteria from infected mice\(^{29}\).

As mentioned above, anti-urease antibody responses to infection are qualitatively distinct from those resulting from artificial immunization. Infected animals do not develop detectable IgA antibodies in secretions, whereas those immunized with rUrease and LT develop strong secretory IgA antibodies that persist for months. Whether this distinction applies also to humans is of interest. While the human response to primary immunization with urease has not been determined, it is clear that natural infection with *H. pylori* results in highly variable serum IgG and serum and secretory IgA antibody responses to urease (OraVax, unpublished data). These data suggest that eliciting a consistent high-level anti-urease response through immunization may increase host resistance to *Helicobacter*.

**Feline efficacy studies**

Mice are highly immunoresponsive and may not provide a predictive model for human immunization. Recently Fox and colleagues\(^ {30}\) have described the susceptibility of cats to *H. pylori*, providing the opportunity to extend vaccine studies to a non-murine host. To determine the immunogenicity of rUrease and to assess its protective efficacy against challenge with *H. pylori*, two groups of 4 domestic cats, predetermined to be free of infection by culture and serology, were orally immunized once weekly for 4 weeks with 10 mg rUrease + 25 μg LT or with LT alone\(^ {31}\). Three weeks after the last immunization, all cats receiving vaccine had a significant rise (> 8-fold) in serum IgG and salivary IgA anti-urease antibodies. Immunohistochemical samples collected 5 weeks after immunization showed the presence of IgA- and urease-specific antibody-containing cells in the antrum and duodenum of cats receiving vaccine, but not in cats receiving LT alone. Fourteen weeks after the last immunization, the cats were challenged with a human-derived CagA+ *H. pylori* strain. Approximately 2 months after challenge, the animals were euthanized and quantitative *H. pylori* cultures performed on 10 gastric biopsy samples per animal. While *H. pylori* infection was not completely prevented, the bacterial density was significantly lower in urease-vaccinated cats than in the control cats (median 147 CFU *versus* 3,226 CFU, respectively, \(P = 0.043\), Wilcoxon rank sums test) Histopathological evaluation also showed a trend toward resolution of inflammation in the corpus and cardia. At sacrifice,
urease-specific IgA in gastric secretions and urease-specific antibody containing cells in gastric mucosa were found in cats receiving urease and LT, but not in those receiving LT alone, confirming observations in mice that artificial immunization, but not natural infection, results in gastric slgA responses.

**Therapy in a Helicobacter model of infection**

Treatment of *H. pylori* infection in patients with peptic ulcer disease is now an accepted health practice in the US and Europe. However, antimicrobial therapy has a number of inherent limitations that might be overcome by use of an effective vaccine or a combined regimen of antibiotics and vaccine. On average, primary treatment failures occur in approximately 15% of patients treated with antibiotics combined with an antisecretory drug. Poor compliance with complex antibiotic regimes and antibiotic resistance in *H. pylori* contribute to treatment failures. In contrast to antibiotic treatment, vaccine-induced immunity would not be expected to select for resistant or more virulent organisms. Since immunological mechanisms are distinct from those involved in antimicrobial treatment, vaccines alone or synergistic activities of vaccines and antimicrobials could potentially achieve the ultimate goal of 100% cure.

Therapeutic activity has been documented in mice using recombinant urease and crude cell antigens, with efficacy rates determined by gastric urease activity between 50 and 94%. When vaccine and a partially-effective antibiotic regimen were combined, the latter proved to be more effective than either treatment alone. These studies were conducted in mice with sub-chronic *H. felis* infections, the immunization regimen being applied only a few weeks after infecting the animals, and it is uncertain whether treatment would be as effective in a chronically infected host. When the *H. pylori* mouse model was employed and therapeutic activity of urease-LT immunization was measured by quantitative culture, a 10-fold reduction in bacterial density was observed, which, however, was highly significant (*P* = 0.0016).

In ferrets, immunization with urease and CT adjuvant resulted in presumptive cure of truly chronic *H. mustelae* infections. When tested 6 weeks after immunization, 30% of ferrets were cured of their infection. A significant reduction in gastric inflammation was demonstrated by histopathology in up to 60% of animals. Interestingly, gastric inflammation was significantly reduced in the cured and persistently infected vaccinated animals compared with infected controls, a finding similar to that described in rhesus monkeys. The possibility that vaccines could diminish the pathologic consequences of *Helicobacter* infections thus deserves further study.
A study was performed by Lee et al. to determine the protective activity of rUrease in an experimental rhesus monkey challenge model. Since most adult rhesus monkeys have pre-existing infections, animals with gastroscopically confirmed infections were immunized. Therapeutic activity was determined by follow-up gastroscopies, and animals remaining infected were cured by administration of antibiotics and subsequently challenged with *H. pylori* to define protection against reinfection. The initial immunization regimen employed 6 doses of urease and LT administered orally over an 8 week interval; controls were sham-immunized with LT alone. Urease-specific IgG antibodies were generated in the serum in 5 of 6 animals and IgA antibodies in the saliva in 3 of 6 animals. None of the immunized monkeys cleared their infections as a result of vaccination. The 6 animals receiving LT only and 5 surviving animals that had received urease and LT were, therefore, treated with antibiotics, omeprazole and bismuth. *H. pylori* was eradicated in all 11 animals as determined by culture and histology of multiple gastric biopsies 5 weeks and 4 months after treatment. The animals then received a single booster dose of vaccine (controls received LT alone) and were subsequently challenged with *H. pylori*. Biopsies taken from the gastric antrum and corpus 3 weeks after inoculation showed a decrease in the level of *H. pylori* colonization in animals receiving urease-LT (a median value of 15 CFU) compared to animals receiving LT alone (median 1,068 CFU, P = 0.047 Wilcoxon rank sums test). This study provided the first evidence in a primate host for reduction in bacterial density due to vaccination.

Clinical trials

Clinical testing of recombinant urease was initiated by our group in 1994. Our clinical studies were carried out in healthy, infected volunteers because of concern that immunization of naive individuals may potentiate inflammation upon subsequent infection, a phenomenon at that time observed in mice, but subsequently not observed in either cats or monkeys. In addition, because the immune correlates of protection remain undefined, it was felt that the direct measurement of a therapeutic effect in infected subjects would have the greatest clinical significance.

A limited study was first performed to demonstrate the safety and tolerability of oral administration of urease without a mucosal adjuvant. In a randomized, double-blind, placebo-controlled trial conducted by Kreiss and colleagues, six infected asymptomatic adults were administered a total of four doses of vaccine, each dose consisting of 60 mg of recombinant *H. pylori* urease administered by the oral route once a
Helicobacter infection

week. Six infected subjects received placebo. As expected in the absence of an adjuvant, none of the vaccinated individuals mounted an immune response, and gastric biopsies obtained before and one month after vaccination showed no changes in bacterial density (measured by quantitative culture), inflammation or mucosal damage. No adverse events were attributable to administration of urease.

A second trial was conducted to determine the tolerability of co-administration of urease with a mucosal adjuvant (LT) in healthy, adults with *H. pylori* infections, and to obtain preliminary data on therapeutic activity. Preliminary results of this trial, which was conducted at the Centre Hospitalier Universitaire, Lausanne and at the Center for Vaccine Development, University of Maryland, were reported by Michetti *et al* at the Helicobacter congress in Copenhagen in 1996. Native LT purified from *Escherichia coli* had been supplied by the Naval Medical Research Institute, Bethesda, which had previously reported adjuvant activity in a study involving cholera vaccine. The controlled trial involved administration of four weekly, graded doses of urease (20, 60, or 180 mg) with LT; placebo vaccine with LT; or placebo vaccine and placebo adjuvant to groups of 4–5 volunteers. The ELISPOT assay for antibody-secreting cells (ASC) in peripheral blood was the most sensitive determinant of immunological responses to the vaccine; 6 of 14 (43%) subjects who received urease, but none of the 10 subjects who received placebo vaccine, had an increase in IgA or IgG ASC at one or more time points, measured 7 days after each successive dose of vaccine. Gastric biopsies were obtained before and 1 month after completion of the immunization regimen. Differences were determined between pre- and postimmunization *H. pylori* densities in gastric mucosa. While the urease-treated groups were not significantly different from control groups with respect to the change from baseline to postimmunization, the subjects receiving active urease experienced, on average, a larger decrease in bacterial densities from baseline to postimmunization (*P* = 0.032) than did those subjects receiving placebo (*P* = 0.425). While the study had small sample sizes per group and was not powered to detect significant differences between treatment groups, it provided the first clinical evidence for a therapeutic activity of oral urease with LT adjuvant.

**Conclusions and future research**

*H. pylori* is a human infection with grave disease consequences, that could be prevented through immunization. A convincing body of data now exists supporting the potential for successful immunization against *H. pylori*. The complex pathogenesis of this infection, including the
Helicobacter pylori and non-ulcer dyspepsia

presence of antigens on H. pylori shared with the host (a mechanism for immune evasion), demand novel approaches for the development of a final vaccine formulation. The selection of defined and well characterized recombinant sub-unit antigens appears to be the most viable approach, and the urease antigen has so far proved most potent in eliciting protective immunity. It is reasonable to assume that more than one protective component will be needed in a vaccine, and a number of such antigens in addition to urease have now been discovered. In addition to antigen composition, a successful vaccine must be delivered to the host in a manner that elicits protective immunity, particularly at the site of bacterial colonization. Mucosal routes of immunization with a classical mucosal adjuvant (LT) have yielded the best results, but prophylactic/therapeutic activity is still incomplete. Research is needed on the mechanisms of protective immunity induced by vaccines, on the protein-specific immune responses to natural infection, and on the functional role of T cells. Such studies may provide important data leading to novel immunization methods, as well as surrogate tests for protection useful in vaccine trials.

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