Helicobacter pylori vaccine: mucosal adjuvant & delivery systems

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Vaccination, especially mucosal vaccination, is considered to be effective in the management of Helicobacter pylori infections. However, most antigens alone cannot induce immune responses when administered mucosally and need to be co-administered with adjuvants or delivery systems. The current research on the mucosal adjuvant and delivery systems of vaccine against H. pylori, including advantages and disadvantages, mechanisms and applications is discussed in this review. Mutants of cholera toxin (CT) and the heat labile enterotoxin of Escherichia coli (LT), CpG oligodeoxynucleotides, biocompatible and biodegradable polymers, and live attenuated bacterial vectors may be promising adjuvant and delivery systems for H. pylori vaccine.

Key words Adjuvant - delivery systems - Helicobacter pylori - vaccine

Helicobacter pylori, a Gram-negative, microaerophilic, spiral-shaped bacterium, whose primary niche is the human gastric mucosa, is the major cause of chronic gastritis and peptic ulcer and plays a role in the development of gastric carcinoma. In 1994, the bacterium was categorized as a class I carcinogen/definite human carcinogen by the World Health Organization. H. pylori colonizes the stomach in about 30-50 per cent of all humans in countries with high socio-economic standards, but in developing countries more than 80 per cent may be infected. In addition, seroprevalence studies indicate that H. pylori infection is also associated with cardiovascular, respiratory, extra-gastro-duodenal digestive, autoimmune diseases. Further, H. pylori eradication is recommended in children with non clear causative iron deficiency anemia (IDA) and patients with idiopathic thrombocytopenic purpura (ITP).

The current management of H. pylori infections relies on antibiotic therapy, consisting of a combination with two different antibiotics together with a proton-pump inhibitor with or without colloidal bismuth, which in most cases is successful in eradicating the bacteria. However, this strategy has a number of drawbacks including therapy failure due to emerging resistance, lack of patient compliance, side effects of the antibiotics and high cost of treatment. The most significant drawbacks of antibiotic therapy are its failure to prevent reinfection, and the increasing number of resistant strains; and these are the driving force to develop a vaccine against this infection.

Since the initial studies which demonstrated that it was possible to reduce gastric Helicobacter colonization by vaccination with H. pylori antigen and adjuvant, various approaches including whole cell vaccines, recombinant antigens (e.g., urease A/B subunits, CagA, VacA, NapA, catalase, or heat shock proteins) in combination with bacterial toxins or other adjuvants have been successfully tested. An important
aspect of *H. pylori* vaccine is the selection of antigen. Vaccination trials exploiting the antigenic properties of some proteins, such as urease, the vacuolating toxin (VacA), the cytotoxin-associated antigen (CagA), the blood-group-antigen-binding adhesin (BabA), and the neutrophil-activating protein (NAP), have been done

*H. pylori* infection causes a cellular infiltration of both neutrophils and CD4+ lymphocytes, as well as secretion of proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-\( \alpha \)) and interferon-gamma (IFN-\( \gamma \)), which is indicative of a T helper type 1 (Th1) immune response\(^2\). Although different vaccination protocols in various animal models of infection\(^3\) and human trials\(^4\) have been performed, the mechanism of protection against *H. pylori* infection is still not understood. Data obtained with transgenic mice suggest that MHC-II restricted CD4+ T-cells play a fundamental role in protection\(^5\), and a shift from a T-helper 1 (Th1) cytokine response induced by *H. pylori* infection to a T-helper 2 (Th2) response has been suggested as a possible mechanism\(^6\). Some recent studies have contradicted the Th2 paradigm and demonstrated the importance of the Th1 response cytokines such as interleukin (IL)-12, IFN-\( \gamma \) and IL-18, in an effective protection following vaccination for *H. pylori*\(^7\).

It is obvious that infections caused by microorganisms that gain access to the body via the mucosal membranes are best prevented by mucosal vaccination. The advantages of mucosal vaccination are numerous and include high patient compliance, ease and low cost of application (i.e., no need of trained personnel) and a decrease in the risk of the unwanted needle-borne infections (AIDS, hepatitis, etc.). Further, vaccination at mucosal surfaces may stimulate both systemic and mucosal immunity; the latter not only at the site of vaccination, but also at distant mucosal epithelia\(^8,9\). It could also prevent infection by neutralizing the pathogen at the site of entry\(^10\). Because antigens alone are not sufficiently taken up after mucosal administration, these need to be co-administered with adjuvants or delivery systems. This review discusses the current situation regarding research towards the mucosal adjuvant and delivery systems of vaccine against *H. pylori*.

**Adjuvants**

Adjuvants are substances which enhance the ability of antigen to elicit an immune response\(^11\). Since the discovery of the adjuvant activity of aluminum compounds over eight decades ago\(^12\), more than 100 empirically derived adjuvants and adjuvant variations have been tested both pre-clinically and clinically\(^13\). Nearly all of these adjuvants failed to win approval for use in routine vaccines due to toxicity concerns. An ideal adjuvant would elicit a persistent, high quality immune response to an antigen while being non toxic, biodegradable, non immunogenic and chemically defined for reproducible manufacture\(^14\). A number of mucosal adjuvants such as aluminum hydroxides\(^24\), Freund’s adjuvant\(^25\), cholera toxin\(^26,27\), *Escherichia coli* heat-labile enterotoxin\(^28,29\), DNA sequences\(^30,31\) and many others have been investigated in order to improve the immune responses to antigens delivered mucosally for *H. pylori* vaccine.

**Aluminum hydroxides:** Aluminum hydroxides Al(OH)\(_3\), are the most widely used vaccine adjuvants with decades of clinical safety experience. Intraperitoneal immunization with an alum adjuvanted vaccine provides not only protection, but also eradication of the already transmitted *H. pylori*. Furthermore, parenteral immunization with Al(OH)\(_3\) has been shown to induce a local decrease of the Th2 cytokine IL-10 and local increase of the Th1 cytokine IL-12 in the stomach\(^32\). Gottwein *et al.*\(^33\) showed the contrary results. A vaccine (HP3) consisted of sterile purified recombinant *H. pylori* vacuolating cytotoxin A (VacA), cytotoxin-associated antigen (CagA), and neutrophil-activating protein (NAP) given intramuscularly with aluminium hydroxide as an adjuvant to non infected healthy subjects demonstrated well-tolerated and strongly immunogenic, generating specific antibody and T-cell responses. However, it has come under scrutiny due to concerns about aluminum related macrophagic myofascitis\(^34\) and the potential for cumulative aluminum toxicity which has been associated with amyotrophic lateral sclerosis, Alzheimer’s disease and dialysis-associated dementia\(^35\).

**Freund’s adjuvant and its incomplete form:** Another conventional adjuvants include complete Freund’s adjuvant (CFA) and incomplete Freund’s adjuvant (IFA). Both IFA and CFA provided partial protection from a challenge with infectious *H. pylori* when the vaccine was administered subcutaneously\(^36\). Neonatal immunized mice also had reduced bacterial loads when immunized intraperitoneally with CFA, and the use of CFA and IFA induced mixed Th1-Th2 responses with a strong IFN-\( \gamma \) component\(^37\). Otherwise, protection could be achieved by the induction of a Th1 cytokine-mediated immune response as generated through the use of CFA\(^38\).


Cholera toxin (CT) and Escherichia coli heat-labile enterotoxin (LT) and mutants: The two mucosal adjuvants used in the majority of immunization experiments to date are cholera toxin (CT) and the heat labile enterotoxin ofEscherichia coli (LT) \textsuperscript{36,45}. In some previous experiments, CT and LT were used as adjuvant for immunization ofmice and rhesus monkeys\textsuperscript{36,39,46,47}. The mucosal adjuvanteffects of CT and LT include an increase in epithelium permeability, the modulation of antigen presentation and ofco-stimulatory molecule expression by antigen presenting cells\textsuperscript{48,49}. Isotype differentiation of B-cells to IgA production, induction of T-cell proliferation andcytokine production can also be observed\textsuperscript{50}. CT induceda strongly polarized Th2 response\textsuperscript{51} and LT induced a morebalanced Th1/Th2 response\textsuperscript{52}, these fit with theobservation that a shift from a polarized Th1 towards aTh0 or Th2 immune response is necessary for protectionagainst infection with H. pylori\textsuperscript{53,54}. However, theseadjuvants are unacceptable for human use due to their high toxicity. LT that was used as an oral adjuvant inhumans is associated with cramping and diarrhoea\textsuperscript{20}. CT has proven to be effective in many animal models, butits toxicity and allergenicity are much higher in humans.  

CT is able to translocate to the olfactory bulb via theolfactory epithelium after intranasal administration,leading to inflammatory responses in the central nervoussystem\textsuperscript{55,56}. To circumvent such issues, geneticallymodified derivatives obtained by site-directedmutagenesis have been constructed\textsuperscript{38,57}. This strategyhas met with some success and is currently under furtherdevelopment.

Mutants of E. coli heat labile enterotoxin such as LTK63\textsuperscript{57}, LTR192G\textsuperscript{38}, LTR72, have been tested inanimal experiments. Among these adjuvants, LTK63 (serine-to-lysine substitution at position 63 in the Asubunit), totally devoid of toxic activity in vitro and in vivo\textsuperscript{38}, is active as mucosal adjuvant in differentanimal models and enhances protective efficacy inappropriately animal models of challenge. Further,LTK63 preferentially polarizes the cellular immuneresponses against the co-administered antigens toward aTh0/Th1 functional phenotype\textsuperscript{59}. LTR192G hasbeen tested in clinical trials and shown to enhance theimmunogenicity of vaccines at the mucosal level\textsuperscript{38,19}.

CpG oligonucleotides: Synthetic oligodeoxynucleotidescontaining immunostimulatory unmethylated CpGmotifs, which are less reactiveogenic than CT, wereinvestigated as a new candidate for H. pylori vaccine adjuvant. Bacterial DNA contains specificimmunostimulatory motifs that can trigger an innateimmune response through toll-like receptor-9 (TLR-9). Specific motifs of CpG oligonucleotides ODNs canvary in their ability to induce individual immune effects,depending on variations in the flanking sequences\textsuperscript{60,61}.Immunostimulatory oligodeoxynucleotides (ISS-ODNs) areactively phagocytosed by cells of the immune system andbind to intracellular receptors present on phagosomes. Stimulation of plasmacytoidDCs can trigger the activation of CD4+ cells via expression of MHC II, but also the activation of CD8+T cells by expression of MHC I. Injection of ISS ODNscan also lead to increased activity of natural killer (NK)cells, resulting in IFN secretion and lysis of cells\textsuperscript{62}.CpG ODNs thereby have potent effects on innate andadaptive cellular immune responses.

CpG oligodeoxynucleotides are known for theirability to induce nearly entirely Th1-biased immune responses and may be approved for human use infuture\textsuperscript{66,62}. Immunization of mice with H. pylori lysate and CPGinduced high titres of Helicobacter-specificantibodies and a strong local and systemic Th1 immuneresponse. Despite this strong Th1 response, mice were notprotected from infection with H. pylori yet had a 10-fold reduction in the number of H. pylori in thegastric mucosa compared to non immunized mice. Also reduction of the bacterial density in immunizedmice was accompanied by a significantly enhancedgastritis\textsuperscript{40}. Intranasal immunization with CpG-oligodeoxynucleotide induced a Th1-type response andproduction of IFN-γ. These all suggest that CpG-oligodeoxynucleotide can be utilized as an adjuvant forH. pylori vaccine.

Delivery systems

For successful vaccine, it is necessary to develop vectorseffective capable of efficiently introducing interest materials into target cells\textsuperscript{64}. The most widelyinvestigated delivery systems are biocompatible andbiodegradable polymers, poly (lactide-co-glycolide) (PLG) microparticles\textsuperscript{64,66}, poly (lactide-coglycolide) (PLGA) microparticles\textsuperscript{65-68}, chitosan (CS)(a bioadhesive polysaccharide)\textsuperscript{66,70}, liposomes\textsuperscript{67} and geneticallymodified whole viruses\textsuperscript{71}.

Polymers: A number of polymers have been investigated ascarriers both for DNA and proteins, including PLGA[poly(D,L-lactic-co-glycolic acid)]\textsuperscript{72}, PLA[poly(D,L-lactic-co-glycolide)] and chitosan. Theefficacy of microparticles following mucosal deliveryis, at least in part, a consequence of their uptake intothe intestinal Peyer’s patches (PBs) following oral
administration or the nasal-associated lymphoid tissue (NALT) following intranasal administration\textsuperscript{73,74}. Microparticles smaller than 10 μm are taken up by the M-cells and transported to the dome of the Peyer’s patches. Most microparticles larger than 5 μm stay in the Peyer’s patches, while microparticles smaller than 5 μm are transported through the efferent lymphatics to the spleen and lymph nodes, where specific IgM and IgG are produced\textsuperscript{75}. The potential for immunization is dependent on the physicochemical and biological characteristics of the system. In microparticulate systems, the particle size and release profiles of antigens play an essential role in the immunization potentials.

PLGA [poly(D,L-lactic-co-glycolic acid)] and PLA [poly(D,L-lactide-co-glycolide)] microparticles-Several kinds of poly (D,L-lactide-co-glycolide) microparticles containing \textit{H. pylori} whole-cell lysate (PLG-HP) could stimulate the \textit{H. pylori}-specific mucosal and systemic response \textit{in vivo} and might be useful adjuvant in future \textit{H. pylori} vaccine development. All types of PLG-HP microparticles have induced higher antibodies production (approximately 4–10 times) than those with soluble antigen in serum and gut washing fluids\textsuperscript{76}. Although PLG microparticles effectively induced the production of anti- \textit{H. pylori} specific IgA antibody in gut washing fluids as well as IgG antibody in the serum, antibody levels were less than those of CT after repeated immunization. PLG-HP microparticles, which are safer to use in human than CT, could stimulate \textit{H. pylori}-specific mucosal and systemic immune responses in mice and may be useful in future \textit{H. pylori} vaccine development.

The adjuvant system of most microparticles is considered to function on the principle of efficient phagocytosis and transport to the lymph nodes and sustained antigen release over extended time periods which may present a continuous trickle of antigen to the immune system\textsuperscript{77}. Probably because of their (pseudo) lipophilic character, these microparticles have proven to be taken up by the PPs\textsuperscript{76}.

This delivery system also has disadvantages. Antigen incorporation requires the use of organic solvents, which may denature the antigen. Upon hydrolysis of the polymers, the pH decreases strongly, which also may deteriorate the antigen.

Chitosan microparticles - Chitosan [a(1-4) 2-amino 2-deoxy β-D glucan] is a linear polysaccharide derived from partial deacetylation of chitin, the major compound of exoskeletons in crustaceans and the second most abundant natural polysaccharide in nature. Chitosan is a mucoadhesive polymer that is able to open tight junctions and allow the paracellular transport of molecules across mucosal epithelium and is therefore suitable for the mucosal delivery of vaccines\textsuperscript{78,79}.

Since cationically charged chitosan can be complexed with negatively charged plasmid DNA and promising results with chitosan as a gene delivery carrier have been obtained\textsuperscript{80,81}. Chitosan can effectively bind DNA and protect DNA from nuclease degradation. On the other hand, sonication and organic solvents are not used for the preparation of chitosan microspheres. This suggests that process is mild enough not to inflict any damage on the DNA. DNA-loaded chitosan microparticles were found stable during the storage. Thus it appears to be a good candidate for the gene delivery system. Chitosan is shown to be a promising candidate in mucosal vaccine delivery for protein vaccine, such as \textit{Bacillus anthracis}\textsuperscript{82}, diphtheria\textsuperscript{83}, influenza\textsuperscript{84}, and \textit{Toxoplasma gondii}\textsuperscript{85}. In view to reduce the cost, chitosan can be used to replace the expensive polymer (PLGA) since both PLGA and chitoan microsphere based formulations produce an equal immune response.

Our previous studies showed that \textit{H. pylori} vaccine with chitosan as adjuvant could protect against \textit{H. pylori} infection and induce both Th1 and Th2 type immune response\textsuperscript{70}. Further, it could promote Th1 and Th2 immune response, reverse the inhibition of Th2 induced by \textit{H. pylori} infection and recover the Th1/Th2 imbalance, which might contribute to the immune protection against \textit{H. pylori}\textsuperscript{86}. We also showed that the level of sIgA in gastric mucosa and specific anti-Hp IgA in the groups with chitosan as adjuvant was comparable in the groups with CT as adjuvant\textsuperscript{87}.

\textit{Bacteria and virus live vectors:}

Modified polio virus vector - Genetically engineered poliovirus offers many attractive features for development as a vaccine vector for mucosal immunization. Poliovirus is transmitted orally and enters immunoreactive mucosal sites. Moreover, the virus is available in an attenuated form that is both safe and effective as an oral vaccine\textsuperscript{88}. Poliovirus replicons encoding the B subunit of \textit{H. pylori} urease could elicit a Th1 associated immune response and provide significant prophylactic and strong therapeutic protection against \textit{H. pylori} in mice\textsuperscript{71,89}.

\textit{Salmonella typhimurium} - Live attenuated \textit{Salmonella} vaccine strains expressing foreign antigens are a
promising new generation of vaccines that induce remarkably strong and specific immune responses in the mammalian hosts when given either by the oral, nasal, rectal or vaginal routes. The efficacy of attenuated *Salmonella* as a good vaccine in humans was first demonstrated in studies using a chemically mutated *S. enterica* serovar Typhi Ty2la strain in adult volunteers and later in children in a large field trial in Egypt. A clinical study showed that preimmunized with *S. enterica* serovar Typhi Ty2la (pDB1) expressing subunits A and B of recombinant *H. pylori* urease showed cellular immune responses to *H. pylori* urease (56%). This supports the results of a previous study in non preimmunized volunteers where 56 per cent (five of nine) of the volunteers showed a cellular immune response to urease after immunization with *S. enterica* serovar Typhi Ty2la (pDB1). Promising results have also been shown in studies using *S. enterica* sv. *Typhimurium* in its natural host, the mouse model, and in human studies (Table).

Compared with traditional vaccines, live attenuated *S. enterica* serovar Typhimurium is used as a new type of vector which does not require antigen purification. It only protects antigens from degradation and denaturation in the stomach but also expresses adjuvant activity and prevents oral tolerance. Other advantages of the live attenuated *Salmonella* vaccines include their safety, case of administration, long lasting protection and no adverse reactions in comparison with the former inactivated whole cell typhoid vaccines. As attenuated *Salmonella* spp. can be easily cultured in large quantities, the vaccine can be produced at low cost, which is particularly important for treating people in the developing world where *H. pylori* prevalence varies from 10-90 per cent.

According to the rules stipulated by Food and Drug Administration, drug resistance plasmid cannot exist in live vaccine and the stability of recombinant plasmid cannot be maintained in human beings and animals by antibiotic. In order to express foreign protective antigen in attenuated *S. enterica* serovar Typhimurium strain without antibiotic, the vector-host balanced lethal system has been developed and widely used. We recently constructed a live attenuated *S. enterica* serovar Typhimurium strain harbouring the *H. pylori* BabA2 and *UreI* fusion gene. The BabA2 and *UreI* fusion gene amplified by PCR was inserted into the prokaryotic expression vector pYA3342 containing *asd* gene and introduced into the attenuated *S. enterica* serovar Typhimurium strain x8501 (Δasd) through transformations. The expressed fusion protein showed satisfactory immunoreactivity and immunogenicity. Other attenuated strains of *S. enterica* serovar Typhimurium, such as the *phoPc* strain, attenuated in macrophage survival and avirulent in mice, *phoP/phoQ*-deleted *S. enterica* serovar Typhi Ty2 (Ty800), and the aroA mutant strain have been tested both in animal experiments and clinical trials, and stimulated strong mucosal humoral responses and cell-mediated responses.

Live attenuated *S. enterica* serovar Typhimurium vaccine may have some drawbacks, including difficulties in expressing several different putative protective antigens in the same host organism and ensuring stable production of all these antigens during colonization; risk of developing an immune response against the vector organism and pre-existing immunity preventing colonization of the vaccine strain; and problems of attenuation phenotypic stability and toxicity recovery.

**Table.** Experiments and trials involving *H. pylori* vaccines with *Salmonella* as delivery systems

<table>
<thead>
<tr>
<th>Salmonella strain</th>
<th>Antigen</th>
<th>Route</th>
<th>Year</th>
<th>Experiment or trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated <em>S. enterica</em> Typhimurium X4072 (pYA248-AB)⁹⁷</td>
<td>Conservative region of adhesion AB</td>
<td>Oral</td>
<td>2004</td>
<td>Animal experiment (mice)</td>
</tr>
<tr>
<td>Live <em>S. enterica</em> Typhimurium <em>phoPc</em> bacteria⁹²</td>
<td>The A and B subunits of <em>H. pylori</em> urease</td>
<td>Nasal</td>
<td>1998</td>
<td>Animal experiment (mice)</td>
</tr>
<tr>
<td>Attenuated <em>S. enterica</em> Typhimurium SL3261⁹³</td>
<td>Urease subunit B (<em>UreB</em>)</td>
<td>Oral</td>
<td>2002</td>
<td>Animal experiment (mice)</td>
</tr>
<tr>
<td>Attenuated <em>S. enterica</em> Typhi⁹⁴</td>
<td>Urease</td>
<td>Oral</td>
<td>2000</td>
<td>Clinical trial</td>
</tr>
<tr>
<td>Attenuated <em>S. enterica</em> Typhimurium live vaccine SL3261 strain⁹⁵</td>
<td>Urease subunits A and B</td>
<td>Oral</td>
<td>1997</td>
<td>Animal experiment (mice)</td>
</tr>
<tr>
<td>Live recombinant <em>S. enterica</em> Typhi Ty2la⁹⁶</td>
<td>Urease A and B</td>
<td>Oral</td>
<td>2002</td>
<td>Clinical trial</td>
</tr>
<tr>
<td><em>PhoP/phoQ</em>-deleted <em>S. enterica</em> Typhi⁹⁷</td>
<td>Urease</td>
<td>Oral</td>
<td>2000</td>
<td>Clinical trial</td>
</tr>
<tr>
<td>Attenuated <em>S. enterica</em> Typhimurium SL3261⁹⁸</td>
<td>Urease</td>
<td>Oral</td>
<td>2005</td>
<td>Animal experiment (mice)</td>
</tr>
<tr>
<td>Attenuated <em>S. enterica</em> Typhimurium⁹⁹</td>
<td><em>H. pylori</em> ureB and IL-2</td>
<td>Oral</td>
<td>2007</td>
<td>Animal experiment (mice)</td>
</tr>
<tr>
<td>Attenuated <em>S. enterica</em> Typhimurium strain SL3261¹⁰⁰</td>
<td>Catalase</td>
<td>Oral</td>
<td>2003</td>
<td>Animal experiment (mice)</td>
</tr>
</tbody>
</table>
Liposomes - Oral liposome-encapsulated recombinant *H. pylori* heat shock protein 60 (Hsp60) vaccine was prepared and its effect against *H. pylori* infection in mice was investigated. PBS or liposome alone showed no immune-enhancing effect, and rHsp60 plus CT, liposome-encapsulated rHsp60 and liposome-encapsulated rHsp60 plus CT showed 73.3, 66.7, and 86.7 per cent, respectively protective rates against *H. pylori* infection. The latter 3 preparations significantly reduced *H. pylori* infection and alleviated the inflammation in the gastric mucosa of the mice challenged with *H. pylori*. The oral liposome may serve as a potent adjuvant against *H. pylori* vaccine in preventing *H. pylori* infection\(^\text{114}\). However, the use of cationic lipids in vivo is hindered by it toxicity, complement activation and liver tropism.

**Others**

**Bacterial ghosts:** Ghosts are empty bacterial cell envelopes without cytoplasm and DNA\(^{115}\), which may be generated by the controlled expression of the PhiX174 lysis gene E in Gram-negative bacteria to obtain vaccine candidates. The gene E-encoded protein was suggested to form a transmembrane tunnel in the bacterial cell wall, through which the cytoplasmic contents are expelled\(^{115}\). Bernhardt et al\(^{116}\) suggested that the lysis protein inhibits cell wall synthesis and thus kills the bacteria. Although the mechanism of genetic inactivation is still a matter of debate, the advantage of ghosts is that they share functional and antigenic determinants of the envelope with their living counterparts and thus represent ideal vaccine candidates.

Prophylactic oral vaccination experiments using these *H. pylori* ghosts in the BALB/c mouse model showed a significant reduction of the bacterial load in the group with ghost, as measured by a quantitative bacterial reisolation procedure\(^{117}\). Co-administration of ghosts with CT as mucosal adjuvant resulted in a complete protection of mice against *H. pylori* challenge, with three animals showing a sterile immunity\(^{117}\). **Plants:** Plant made vaccines are specially attractive as plants are free of human diseases, reducing screening costs for viruses and bacterial toxins. In addition, expression of vaccines in plants tissues provides a heat-stable environment, and enables oral delivery, thus eliminating injection-related hazards. Expression of recombinant UreB in rice grain for immunization against infection by *H. pylori* is an attractive system, and UreB was expressed and accumulated in transgenic rice. Rice grains can be utilized to express rUreB as bioreactors for low-cost production and safe delivery of vaccine against infection by *H. pylori\(^{118}\).

In conclusion, the study of *H. pylori* vaccine is a laborious, expensive and time consuming process. Better vaccine formulations, antigen preparation(s), adjuvants, and better delivery systems have to be designed and tested for safety and immunogenicity\(^{10}\). In contrast to one-on-one testing of individual antigen candidates, global techniques such as proteomics provide additional information for identification of vaccine candidate antigens. The proteome of *H. pylori* has been extensively analyzed by several groups affording large data sets of identified proteins\(^{119-122}\). On the other hand, mucosal adjuvant and delivery systems could influence the development of *H. pylori* vaccine both qualitatively and quantitatively. The key point of a successful vaccine lies in the delivery of antigen to the immune system. Although different mucosal adjuvant and delivery systems have had varied levels of success, none of these is commercially available. Questions remained to be answered, efficiency remained to be identified, and risks remained to be proved, until the eradication of *H. pylori*.

**References**


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