Helicobacter pylori as a Vaccine Delivery System

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Abstract

For more than 10 years a vaccine against *Helicobacter pylori* has been the elusive goal of many investigators. The need for a vaccine was highlighted when eradication attempts in developing countries were foiled by reinfection rates of 15–30% per annum. In addition, physicians in developed countries were concerned that attempts at total eradication of *H. pylori* would result in widespread macrolide resistance in both *H. pylori* and other important pathogens. Although attempts to produce vaccines against *H. pylori* have failed in their ultimate goal, considerable knowledge has been developed on the pathogenesis and immunology of *Helicobacter* infections. In this article we describe an alternative use for this new knowledge, i.e. a plan to use live *Helicobacter* species to deliver vaccines against other organisms. Because of its intimate attachment to the gastric mucosa and long-term residence there, *H. pylori* might succeed as an antigen delivery system, a goal which has eluded most other strategies of nonparenteral vaccination.

The need for vaccination against *Helicobacter pylori* infection became apparent only after studies where reinfection rates showed that the initial infection did not protect from future infections. As a result, in countries where reinfection rates were high, only the most severe clinical cases of bleeding peptic ulcer were worth treating with antibiotics. In addition, it was apparent that eradication programs to protect the population from stomach cancer could never be cost effective in countries such as Peru [1] where the infection appeared to contaminate the drinking water.

Since 1993, several vaccines have been developed in animal models that protect from or cure *Helicobacter* by oral or nasal administration of antigens such as urease, CagA, flagellar protein, and heat shock protein [2–5]. Protection can also be partially induced by the transfer of passive antibodies [6]. Alas, rather expensive studies in monkeys [7] and in humans [8,9] showed that these vaccines did not affect gastric colonization with *Helicobacter* in any important way. This outcome might have been expected as in vitro studies show that the immune response to most vaccine antigens is very similar to the natural response to *Helicobacter* infection, a response that seems unable to eradicate or protect. More recently, protection experiments using *Helicobacter* antigens expressed on *Salmonella* sp. have been described [10], but again, protection in humans has not yet been shown.

As these data were being generated, epidemiologic studies showed that humans and *H. pylori* had coexisted for at least 50,000 years, ever since humans had walked out of Africa [11]. This knowledge has led some to postulate that, at least in the Stone Age, *H. pylori* infection might have provided some extra survival value to ancient man, more like a commensal than a pathogen [12]. Perhaps in keeping with this concept, *H. pylori* is not adequately sensed or cleared by the immune system because it does not invade the deep layers of the gastric mucosa. In addition, it may be able to down-regulate the immune system [13] such that lifelong infection and then vertical transmission to offspring is the norm [14]. The success of *H. pylori* as a gastric colonizer is emphasized by the observation that at least half of the population of the whole world is asymptomatically infected [15].

In the past decade the potential threat of global pandemics with new and old pathogens has been recognized. Globalization of trade has increased the risk of disease transmission between countries, so that even sporadic cases of H5N1 avian influenza cannot be ignored in a global context.

Indeed, just as interest in vaccines was waning, the 2003 SARS epidemic made it evident that new pathogens are emerging and novel vaccine strategies are required. Live, recombinant bacterial vaccine vectors are one such strategy. The development of live bacterial vectors for vaccination has been under way for at least 30 years [16], the most well known being attempts to vaccinate against typhoid based on attenuated *Salmonella*. Bacterial vaccinations are potentially cost effective but their safety requires the right balance between antigenicity and pathogenicity. In this respect, *H. pylori* as a live bacterial vector for vaccination could have a major advantage. Because billions of people are infected, mostly asymptomatically, it already has a remarkable safety profile. In addition, *H. pylori* has never presented itself as a significant pathogen in immunosuppressed persons, such as those with HIV [17]. Furthermore, although *H. pylori* is known to cause overt disease in a minority of persons, both peptic ulcer and gastric cancer are relatively uncommon in strains without toxin [18–20]. Finally, as gastric cancer usually occurs after decades of inflammation [21], the possibility of using short-term *H. pylori* infection in vaccination remains a viable option.

Unique Relationship between *H. pylori* and the Gastric Mucosa

Initially, *H. pylori* is likely to be acquired in early childhood via the fecal–oral or oral–oral route from parents or siblings [22]. Small numbers of bacteria arriving in food or drink show negative chemotaxis to stomach acid and, by means of their various attachment proteins, are able to adhere initially to the mucus layer. The urease of *H. pylori* protects the organism from gastric acid long enough to transverse the mucus layer and ultimately some bacteria adhere to epithelial cells of the gastric mucosa. Once adhering to the gastric mucosa the bacteria can freely divide, so that within 3 days they cover the gastric epithelial surface and penetrate the gastric glands. Most interesting is the extreme thickness of the mucus layer, which is normally approximately 50–170 μ thick [23], i.e. 10–20 epithelial cell depths. *H. pylori* normally resides in the area immediately adjacent to the epithelial cells with a minority of the organisms actually adhering to cells.

The lumen of the stomach maintains an acid pH, between 1.5 and 2, except during meals when the pH is temporarily neutral, but is quickly acidified. Because of its urease acting in the lower reaches of the mucus layer, most of the gastric mucus contains high levels of urease, which serves to destroy the urea normally residing in the gastric juice. Consequently, after its initial colonization, resident *H. pylori* probably makes it difficult for new *H. pylori* strains to reach the gastric mucosa and colonize. Thus, even in countries where most people are infected with *Helicobacter*, most of these are only colonized with a single strain [12].

Because *H. pylori* is microaerophilic, it does not survive well in the anaerobic lumen of the stomach or the small intestine, and similarly may find difficulty in surviving beneath the epithelium where oxygen levels are high. The actual microaerophilic nature of *H. pylori* has not well been studied but it may be unable to cause systemic infections because of this preference. For example, there are no reports of invasive *H. pylori* infections or systemic infections, even though many immunosuppressed persons in the world must be infected with the bacterium. In most infections, vast numbers of organisms are present on the gastric mucosa, far more than are usually tolerated with other pathogenic bacteria/organisms. Because the gastric mucosa is an impermeable rather than an absorptive epithelium, such intensive colonization of the mucosa is not necessarily detrimental to the host.

H. pylori and the Immune System

The gastric lamina propria contains few immunocompetent cells. This may be because the mucosa has to stretch in order to accommodate a meal whilst maintaining its integrity as a barrier to acid. This purpose might not be compatible with the type of eradicative immune response which is possible in the small intestine where luminal contents are more benign and only very low numbers of organisms are normally present. The major difference between the two locations is that gastric contents are static for several hours, potentially allowing putrefaction to occur if acid is not present. In the small intestine, however, peristalsis causes fairly rapid transit of acidic food leaving the stomach so that overall bacterial counts remain low.

The relationship between *H. pylori* and the gastric mucosa is shown in Fig. 1. After colonization and attachment to the epithelial cells, *H. pylori* usually secretes the CagA protein via a type IV secretion system, directly into the epithelial cells, which then change their morphology to a more primitive form and even become apoptotic. In addition, CagA compromises the tight junctions between the epithelial cells, and some cells disappear from the mucosal layer, forming a microbreak and allowing *H. pylori* to attach to the basolateral parts of epithelial cells and access the basement membrane. In this position, *H. pylori* can deliver soluble antigens and toxin into the lamina propria. Ultrastructural studies easily demonstrate antigenic components of *H. pylori*, and sometimes even bacterial components phagocytosed within macrophages [24]. Thus, there can be no doubt that the immune system eventually does sense the presence of *H. pylori* on the gastric mucosa. Persistent stimulation of the innate immune response results in continued attraction of

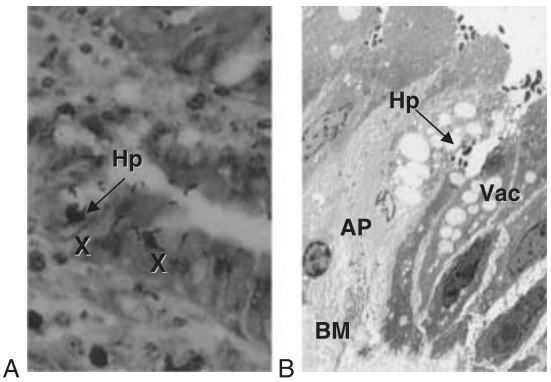

Figure 1 Relationship between *Helicobacter pylori* and the gastric mucosa. (A) Shows several areas where *H. pylori* has penetrated into a "micro ulcer" and is close to the basement membrane (silver stain, 500×). (B) Electron microscopy of an epithelial lesion shows vacuolated and apoptotic cells, with *H. pylori* exploiting the "micro ulcer". Since tight junctions have been breached, soluble *H. pylori* antigens have access through the basement membrane into the lamina propria where they may stimulate an immune response (EM, 3000×).

neutrophils, which migrate through the epithelium and attempt to phagocytose the organisms [24]. Indeed, in mouse models [25] dendritic cells are present in the gastric mucosa once chronic inflammation has been established. Although these studies were performed using *Helicobacter felis*, the model does serve to show that during *H. pylori* infection all the components of mucosal immune system are eventually present.

Whereas the gastric mucosa is initially largely devoid of immune cells, the mature appearance of *H. pylori* infection is one of active chronic gastritis. This means that the lamina propria is infiltrated with mononuclear cells, lymphocytes, plasma cells, and macrophages.

Papers describing infection with *H. pylori* have documented cytokine patterns consistent with the Th-1 type response (cell mediated) or Th-2 (humoral response), or even a mixed response [26]. In the chronic form, *H. pylori* causes a standard immunologic response, inducing IgM, IgA, and IgG production [27,28]. In recent studies, it has been proposed that eradication of *H. pylori* might be possible if the normal immune response was switched to an alternative emphasis, i.e., Th-2 to Th-1, or vice versa. However, it is clear that in the normal situation *H. pylori* provides some negative feedback onto the mucosal immune response to avoid maximal acute inflammation, an event that could cause ulceration and be deleterious to both the host (causing death) and also to the pathogen. For example, one way *H. pylori* may dampen the immune response is via its VacA toxin, which leads to apoptosis of mononuclear cells [29].

Because *H. pylori* only attaches to epithelial cells in the stomach and is never invasive, it seems that the immune response to the gastric infection is quite different from the response to an invasive organism such as *Salmonella*.

Development of *H. pylori* as a Vaccine Delivery System

Using molecular techniques, it is now possible to add new proteins to *H. pylori* and to modify existing proteins so that vaccine antigens can be expressed on the organism. It seems likely that many of the outer membrane proteins can be modified to express antigens without seriously impairing the virulence of the *H. pylori* organism. We can say this because the essential genome of *H. pylori* is only about 1000 genes, i.e., approximately 600 genes are not essential for the organism and are expressed in only a subset of successfully colonizing *H. pylori* strains [30]. Therefore, these nonessential genes should be able to be modified to express vaccine antigens.

Once *H. pylori* has been developed as a delivery system, one might ask what types of vaccines would be usefully presented by *H. pylori*. Unlike viral vectors with limited size, bacterial vectors are able to express many proteins on their cell wall and therefore it should be possible to vaccinate against several diseases at once using a single genetically modified organism (GMO). Thus, all of the common diseases and vaccinations given to the pediatric population would be initial prime candidates for expression in *H. pylori*, such as tetanus, mumps, whooping cough, measles, rubella, and chicken pox. To be practical, particularly in a developing country, a vaccine needs to be successful after a single oral dose, with minimal side effects and long-term protection. The difference between the *H. pylori* system and a normal parenteral vaccine is that *H. pylori* infection can be extremely chronic. Therefore, even diseases against which one is presently unable to be vaccinated might be susceptible to vaccination using an *H. pylori* vector. It may be possible to produce a GMO strain of *H. pylori* expressing antigens of microorganisms involved in diseases such as malaria, tuberculosis, HIV, and hepatitis C. One deficiency of current vaccination might be that potentially useful vaccine antigens do not elicit a strong enough immune response to generate a successful vaccination using the normal regimes. Prolonged *H. pylori* vaccination might offer a solution to this problem.

Alternatively, because of the extreme density of bacterial colonization on the gastric mucosa and the ability of *H. pylori* organisms to penetrate the gastric glands as well as to reach the epithelial basement membrane, its soluble components could diffuse into the lamina propria.

Therefore, it might also be possible to deliver pharmacologic agents via *H. pylori*. Because of the number of organisms present with a total wet weight exceeding no more than a few milligrams at any point in time, the amount of pharmacologic agent that would be delivered through the gastric mucosa will no doubt be rather small. However, it might be possible to deliver hormones or cytokines into the gastric mucosa by expressing them in *H. pylori*.

Most live vaccine delivery vectors are attenuated to reduce the risk of associated disease. Virulence factors of *H. pylori* include VacA, c*ag*A pathogenicity island (*cag*PAI), DupA (duodenal ulcer-promoting protein) and OipA (outer inflammatory protein) [31]. Although various types of these genes are present in bacteria causing important gastric diseases (peptic ulcer and gastric cancer), successful strains of *H. pylori* do exist in which these pathogenic factors are not expressed or where they are expressed in a rather benign form. For example, although ulcer formation is associated with CagA [20], clinical studies show that approximately 50% of nonulcer patients also produce anti-CagA antibodies or are colonized with *H. pylori* harboring the *cag*A gene [20,32,33]. In addition, some colonizing strains lack the *cag*PAI or have types of VacA toxin that are not associated with disease. Absence of toxin such as CagA and VacA is not necessarily associated with reduced colonization or chronicity of *H. pylori* infection in animal models. Certainly, humans can carry lifelong infections with toxin-negative strains of *H. pylori*. Thus for the purpose of vaccines, it is possible to choose strains of *H. pylori* in which harmful genes are absent or disabled to some degree. Additionally, it is relatively easy to delete these genes in such a way that they cannot be easily reacquired by the vaccination organism. Perhaps because *H. pylori* is the only organism colonizing the human stomach it rarely, if ever, acquires antibiotic resistance genes from other organisms, as its environment is not rich in foreign DNA.

In summary then, *H. pylori* may offer a solution to many of the difficult problems confronting vaccine strategies. The "holy grail" is a recombinant bacterial vaccine, which has a number of characteristics: to induce protective immunity within a short period post vaccination, be safe for administration without risk of causing disease in the vaccinee, induce a response that allows serologic differentiation of vaccinated from infected vaccinee, be stable for long-term storage, not be able to be released and survive in the environment, and be traceable in a population and the environment [34]. An *H. pylori*-based vaccine may be able to be administered in a single dose orally, not requiring a booster, be effective against several pathogens and the use of an attenuated vaccine strain would provide balance between protection and pathology. *H. pylori* and other live bacterial vectors are inherently inexpensive, and, depending on the technology required for scale up, can probably be produced inexpensively even in developing countries. In any vaccine strategy, distribution and administration are sometimes a much greater cost than the actual production of the vaccine. Therefore, local manufacture might be an important advantage.

The Ondek Project

Ondek is a company based in Western Australia, which has been created specifically for the purpose of generating vaccines from *H. pylori*. The name Ondek is meant to resemble the words "on deck," suggesting that *H. pylori* may be the vessel within which vaccines are delivered (on the "deck"). Thus, an *H. pylori* platform might be used for many purposes. Initially, this might be vaccination, but subsequent applications could include drug delivery through the gastric mucosa and immunomodulation. Ondek's scientific team is housed within the *H. pylori* laboratory at the University of Western Australia.

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