SCIENCE ALERT

Eradication of chronic *Helicobacter pylori* infection by therapeutic vaccination

Ghiara P, Rossi M, Marchetti M, *et al*. Therapeutic intragastric vaccination against Helicobacter pylori in mice eradicates an otherwise chronic infection and confers protection against reinfection. *Infect Immun* 1997;**65**:4997–5002.

Abstract

Chronic infection of the gastroduodenal mucosae by the gram-negative spiral bacterium *Helicobacter pylori* is responsible for chronic active gastritis, peptic ulcers, and gastric cancers such as adenocarcinoma and low-grade B-cell lymphoma. The success of eradication by antibiotic therapy is being rapidly hampered by the increasing occurrence of antibiotic-resistant strains. An attractive alternative approach to combat this infection is represented by the therapeutic use of vaccines. In the present work, we have exploited the mouse model of persistent infection by mouse-adapted *H. pylori* strains that we have developed to assess the feasibility of the therapeutic use of vaccines against infection. We report that an otherwise chronic *H. pylori* infection in mice can be successfully eradicated by intragastric vaccination with *H. pylori* antigens such as recombinant VacA and CagA, which were administered together with a genetically detoxified mutant of the heat-labile exterotoxin of *Escherichia coli* (referred to as LTK63), in which the serine in position 63 was replaced by a lysine. Moreover, we show that therapeutic vaccination confers efficacious protection against reinfection. These results represent strong evidence of the feasibility of therapeutic use of VacA- or CagA-based vaccine formulations against *H. pylori* infection in an animal model and give substantial preclinical support to the application of this kind of approach in human clinical trials.

Comment

Effective antibiotic based therapies for eradicating *Helicobacter pylori* have been developed in recent years.[1 2] There is, however, an the increasing problem of antibiotic resistance in *H pylori*[3] and in the long term the consequence of large scale eradication programmes could be a reduction in the efficacy of current antibiotic based regimens.

The development of a vaccine against *H pylori* which confers long term protective immunity is the best strategy to circumvent the problem of antibiotic resistance and to eradicate *H pylori* on a global scale. The feasibility of inducing protective immune responses to helicobacter by oral vaccination with bacterial antigens and a mucosal adjuvant was initially demonstrated in the *H felis* murine model.[4–6] Vaccination with both *H pylori* urease[5] and heat shock proteins (HspA and HspB)[6] protected against subsequent challenge with *H felis*. However, *H felis* lacks many of the virulence factors present in *H pylori*, such as the *cag* pathogenicity island[7] and the cytotoxin VacA,[8] precluding analysis of these antigens as candidate vaccines in the *H felis* model. The development of mouse adapted *H pylori* strains which cause chronic infection in mice was a major advance.[9] The *H pylori* mouse model has permitted the testing of vaccines containing purified *H pylori* antigens against homologous challenge infection.[9] To date, a number of protective *H pylori* antigens have been identified which confer immunity against *H pylori* infection in the mouse, including purified VacA,[9] urease,[9] CagA,[10] and catalase.[11]

The prophylactic vaccination studies in animals showed that, in contrast to natural infection, protective immune responses to gastric helicobacter can be induced. The question was then addressed whether vaccination could be used to eliminate existing infection. Oral immunisation with helicobacter sonicates[12] or recombinant urease B subunit[13] together with cholera toxin eliminated chronic *H felis* infection in mice and protected against subsequent *H felis* challenge.[13] Therapeutic immunisation also was successful in ferrets infected with *H mustelae*.[14]

In their study Ghiara *et al* investigated the feasibility of the therapeutic use of *H pylori* antigens as vaccines against chronic *H pylori* infection. Importantly, they also test the ability of a genetically detoxified mutant of the heat labile toxin of *Escherichia coli* (LTK63)[15] to act as a mucosal adjuvant. Their study shows for the first time that oral administration of either *H pylori* sonicates or recombinant proteins (VacA and CagA), together with LTK63, successfully eradicates *H pylori* infection in mice. The treated mice remained non-infected for at least three months after therapeutic vaccination, confirming long term persistence of eradication rather than suppression of the chronic infection. Importantly, Ghiara *et al* also show that the therapeutic vaccine both eradicates infection and confers protection against subsequent challenge.

Successful mucosal vaccination requires strong adjuvants to improve the poor immunogenicity of co-administered antigens. A key role of mucosal adjuvants is likely to be the stimulation of T helper-2 (Th2) type mucosal responses.[16 17] The inherent toxicity of the mucosal adjuvants cholera toxin and heat labile enterotoxin (LT) has been a major limitation for their use as vaccines in humans. Recent clinical studies in *H pylori* infected human volunteers testing the safety and immunogenicity of recombinant *H pylori* urease showed that the co-administration of LT was associated with a high incidence of diarrhoea.[18] The use of genetically detoxified heat labile enterotoxins such as LTK63[15] is likely to circumvent this problem. LTK63 has a single amino acid substitution (Ser to Lys in position 63) which destroys its ADP ribosylating toxic activity.[15] Non-toxic LTK63 has been used success-

fully as a mucosal adjuvant in animal models to induce antigen specific humoral responses[19] and measles virus specific cytotoxic lymphocyte responses.[20] The demonstration by Ghiara *et al* that the genetically detoxified mutant of the heat labile *E coli* enterotoxin is also suitable for therapeutic oral vaccination against *H pylori* is an important development for its future clinical use.

An understanding of the mechanisms involved in the induction of protective mucosal responses to *H pylori* is important for future clinical use of prophylactic and therapeutic vaccines. As discussed by Ghiara *et al*, the role of the adjuvant in therapeutic vaccination may be to change the nature of the chronic gastric Th1 type tissue damaging response to a Th0 or Th2 protective response. In the *H felis* mouse model stimulation of Th2 responses has been associated with a reduction in both bacterial load[21] and gastric inflammation.[22] Down-regulation of Th1 responses by neutralisation of interferon-γ in *H felis* immunised mice resulted in unmasking of both splenic and gastric interleukin 4 (IL-4) Th2 responses.[22] Adoptive transfer of splenic T cells from mice after immunisation and challenge and of in vitro generated *H felis* specific Th2 cell lines also reduced the bacterial load of *H felis* after challenge in naive recipients.[21] Consistent with these observations, Mohammadi *et al* also found that IL-4 knockout mice had an increased bacterial load of *H felis* compared with wild type controls.[21]

Ghiara *et al*, while confirming that chronic *H pylori* infection in the mouse model induces a Th1 response, did not examine the effector mechanisms contributing to the success of therapeutic vaccination. They speculate, however, that therapeutic vaccination induces activation of Th0 or Th2 responses which in turn trigger bacterial eradication. Current evidence suggests that gastric Th1 responses predominate in humans with chronic *H pylori* infection.[23 24] It remains to be investigated whether the human gastrointestinal responses can be similarly modified. If so, given the availability of non-toxic mucosal adjuvants,[15] therapeutic vaccines may prove to be a novel means of eradicating *H pylori* and a therapeutic alternative to the use of antibiotic based regimens.

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