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TOPIC HIGHLIGHT

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Helicobacter pylori vaccination: Is there a path to protection?

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Abstract

Helicobacter pylori (*H. pylori*) is a pathogenic, extracellular bacterium that colonizes the stomach in approximately 50% of the world population. It strongly interacts with the gastric epithelium and mostly causes asymptomatic gastritis. The colonization of *H. pylori* leads to ulcer development in around 20% of infected patients and may progress to gastric cancer or mucosa-associated lymphoid tissue lymphoma in 1%. Thus, *H. pylori* is the major cause of gastric cancer worldwide. It has been classified as a class I carcinogen by the World Health Organization. Since its discovery in the early eighties by Warren and Marshall, research has been focused on the investigation of *H. pylori* biology, host-pathogen interaction, prevention and treatment. Although *H. pylori* induces a strong humoral and local cellular immune response, the pathogen is not cleared and establishes a chronic infection after encounters in childhood. The ability to colonize the stomach is mediated by several virulence factors that change the host environment, promote adhesion to the epithelium, influence the gastric inflammation and induce immune evasion. *H. pylori* can be eradicated by antibiotic treatment in combination with a proton-pump inhibitor, but efficacy is decreasing. Current therapies are expensive, have side effects and contribute to increasing antibiotic resistance, underlining the need for novel therapeutics.

Key words: *Helicobacter pylori*; Vaccination; Immune response; Dendritic cells; T-cells

Core tip: *Helicobacter pylori* (*H. pylori*) is a carcinogenic pathogen colonizing the human stomach. In the advent of rising antibiotic resistance, it is of major interest to introduce novel therapies. Immunization is a potent candidate although all efforts to generate an effective vaccine have failed as yet. The host-pathogen interaction and especially the immune-modulating capacity of *H. pylori* contribute to this development of resistance to treatment. In this review potential solutions with a focus on the immune response to the pathogen are discussed.

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INTRODUCTION

In the last 20 years substantial effort has been put into the development of a *Helicobacter pylori* (*H. pylori*) vaccine. In different animal models immunization strategies have been tested[1] which led to the reduction of colonization, but few strategies conferred protection in terms of sterilizing immunity[2]. Vaccines were composed of different antigens and adjuvants applied by different routes and delivery systems. Some immunization strategies were tested in humans, but with disappointing results[3]. Although

several vaccines induced antigen-specific immunity there was only one publication that reported a slight reduction of *H. pylori*[4]. This raises several questions regarding successful vaccination against *H. pylori*. What is a protective antigen? Which adjuvant can induce a protective immune response? And finally, what impact has the host-pathogen interaction that modifies the host immune system on a successful vaccine and is it possible to circumvent these influences? These questions will be addressed in this review, with regard to the host immune response against *H. pylori* and the experience of vaccination that we have till now.

IMMUNE RESPONSE AGAINST *H. PYLORI*

An encounter with *H. pylori* leads to the activation of the innate and adaptive immune system. The recognition of antigen induces the activation of antigen presenting cells (APCs)[5]. It is unclear if the APCs responsible for recognition reside in the stomach itself. Recent data indicate a role of APCs located in the Peyer's patches (PPs) in sampling for antigen from dead bacteria[6]. It has been clearly demonstrated that antigen presentation, co-stimulation and cytokine production are controlled by toll-like receptors (TLRs)[7]. Immune recognition of *H. pylori* involves TLR2 and TLR5[8]. The local immune response towards *H. pylori* in the stomach is characterized by the presence of CD4+ T-cells in the gastric lamina propria with a mainly T-helper type 1 (Th1) phenotype[9]. Infection also induces IgG and IgA antibody responses detectable in the serum and in the mucosa[10]. Despite the generation of broad innate and adaptive immune responses, *H. pylori* is not cleared, thus chronically colonizing the stomach.

Dendritic cells in H. pylori infection

The hallmark of dendritic cells (DCs) is their potential to take up and present antigens very efficiently[11]. In the gut several DC subsets can be found. The most prominent CD103+ DCs have the ability to induce regulatory T-cells (Treg)[12] as well as imprinting gut homing properties *via* α4β7 integrin expression[13]. A DC subset expressing CX3CR1, negative for CD103, were described as antigen sampling cells that extend their dendrites into the lumen[14]. These data are still controversial and the CX3CR1 cells are not classified as a true DC subset. Most knowledge of gut-residing DCs comes from the intestine but *H. pylori* is a pathogen of the stomach and interacts with the gastric epithelium. The role of DCs in the gastric tissue is less well defined. On the one hand, no organized lymphoid structures are present, and it is not clear if antigen sampling from the gastric lumen exists. On the other hand, it has been described that recognition of *H. pylori* takes place in the small intestine[6]. The coccoid form of *H. pylori* was found to interact with PP DCs and mice lacking PPs fail to induce gastritis thus indicating the priming site of *H. pylori* within the PPs. Nevertheless, DCs can be isolated from the stomach, but their role in local pathogen recognition has to be evaluated.

Most work on DC-*H. pylori* interaction was performed *in vitro* or *ex vivo*. DCs from human and mouse origin have been proven to induce a pro-inflammatory phenotype. Infection of human monocyte-derived DCs with *H. pylori* led to the maturation of the DCs with increasing expression of MHC Ⅱ and co-stimulatory molecules[5]. Furthermore, IL-12 production was observed, and in co-culture experiments naïve T-cells differentiated to a Th1 phenotype characterized by the expression of interferon (IFN)γ and tumor necrosis factor (TNF)α. Notably, the authors could observe that *Escherichia coli* (*E. coli*) induced this phenotype more efficiently. Similar co-culture experiments were performed by Khamri *et al*[15]. They could observe an increased IL-23 production by the DCs inducing Il-17-producing T-cells. The finding that *H.pylori* induces a less inflammatory phenotype then *E. coli* or *Acetinobacter lwoffi*[16] indicates that *H. pylori* is a weak stimulus or is able to actively suppress the DC activation. Furthermore, DCs activated with *H. pylori* were able to convert naïve T-cells to Treg[17]. Whether the *in vitro* generated DCs mirror the phenotype and activity of the cells in the gut mucosa is not well known.

More recent data that addressed the tolerogenic effect of DCs in *H. pylori* infection was generated in an *in vivo* mouse model. Diphtheria toxin (DT)-dependent depletion of DCs in a CD11c-DTR (DT receptor) transgenic mouse was evaluated in the context of *H. pylori* infection[18]. DC depletion in neonatally infected mice resulted in reduced colonization concomitant with an increase in gastritis and IFNγ-producing T-cells. Applying the DC depletion in a vaccination model also decreased the bacterial burden and promoted vaccine efficacy[19]. This phenotype was associated with more pronounced gastritis and IFNγ/IL-17 production. These results demonstrate that DCs seem to be relevant not only to mount but also to suppress an effective immune response against *H. pylori*.

B-cells in H. pylori infection

The infection with *H. pylori* induces a local and systemic antibody response including IgM, IgG and the mucosal active IgA isotypes[20]. Serum of infected patients is successfully used in *H. pylori* diagnosis including different antigen recognition[21]. In a mouse model it could be shown that B-cell knockout mice are colonized to the same level as control animals after two weeks of infection. However, after two and four months the bacterial burden in the B-cell deficient mice decreased significantly accompanied with CD4+ T-cell infiltration and increased inflammation[22]. These data suggest that the B-cell response may be beneficial for *H. pylori*. Furthermore, the chronic infection with *H. pylori* seems to prevent apoptosis of B-cells indicating a possible influence on the generation of mucosa-associated lymphoid tissue lymphoma[23]. In vaccination experiments it could be shown that B-cells do not participate in the post immunization gastritis, because B-cell knockout mice exhibit similar inflammation after prophylactic vaccination to the controls[22]. Thereby, the efficacy of the vaccine was similar to that seen in wild-

type animals. Thus, antibody responses may not promote protection. Nevertheless, the role of B-cells during vaccination against *H. pylori* should be reconsidered in the context of neutralizing antibodies. Blocking essential factors of *H. pylori* could be a promising strategy in future vaccination approaches.

T-cells in H. pylori infection

T-cells play a major role in protection against *H. pylori* through the adaptive arm of the immune system. Mouse knockout models showed that CD4+ T-helper-cells (Th) are responsible for clearing the bacteria in the stomach, whereas B-cells have a minor contribution to protective immunity after vaccination[24]. CD4+ T-cells consist of different subsets with effector (Th1, Th2, Th17) and regulatory (Treg) properties. After infection with *H. pylori*, antigen uptake takes place in PPs by DCs, where naïve T-cells are located[6]. After activation they acquire intestine-specific migration patterns to reach the stomach, the site of infection, and can confer protection. One crucial homing receptor is the α4β7 integrin. T-cells that do not express α4β7 are not able to protect gastric mucosa against *H. felis* infection, as shown by Michetti *et al*[25] in transfer experiments.

Th1/Th2

The expression of the transcription factor T-bet (T-box 21) and the secretion of the effector cytokines IFNγ, IL-2 and TNFα characterize Th1 T-cells, and account for cell-mediated immunity. This is also defined by antibody-mediated responses of certain subclasses of the IgG antibody family, specifically IgG2a. Th2 T-cells express Gata3 and secrete IL-4, IL-5 and IL-13, supporting humoral immunity, particularly those that are involved in allergy, dominated by the IgG1 antibody isotype. For a long time it was thought that Th2 confers protection against *H. pylori*. This view was changed by findings showing that during *H. pylori* infection a pronounced Th1-type CD4+ T-cell response develops with increasing numbers of mucosal T-cells[26] and the induction of gastritis[27]. Studies in IFNγ and IL-4 deficient mice confirmed the important role of a Th1 immune response and the development of inflammation connected with *H. pylori* infection[28]. Nevertheless, *H. pylori* infection induces a Th1 response which neither eliminates the pathogen nor confers protection against reinfection[29,30]. Immunization studies with predominant Th1 responses, characterized by IL-12 and IFNγ production, indicated that protection is due to a Th1 phenotype[31], with the overall conclusion of a protective role for a Th1-type T-cell response.

TH17

Another recently described effector subset are Th17 T-cells, distinct from Th1 or Th2 cells, characterized by the transcription factor Rorgt and the secretion of a subset of cytokines, *i.e.*, IL-17, IL-22 and IL-26[32-34]. They are most abundant at steady state in gut-associated tissues, particularly the small intestinal lamina propria[35,36], where they are thought to coordinate early mucosal responses to pathogens[37,38]. Specifically, Th17 T-cells have significant roles in protecting the host from bacterial and fungal infections, particularly at mucosal surfaces[39,40]. The coexpression of IL-17 and IL-22 by Th17 cells regulates the production of antimicrobial proteins in mucosal epithelium[41]. On the other hand, several reports show their potential to induce autoimmune tissue injury[42,43]. There is a reciprocal developmental pathway for the autoimmunity inducing effector Th17 and regulatory (Foxp3+) T cells that inhibit the pathogenic Th17 autoimmune cells[44,45].

While transforming growth factor (TGF)β1 is necessary for induction of Th17 differentiation, IL-21 and IL-23 are involved in generation and stabilization of the Th17 cell subset, which express the IL-23 receptor[46-48]. The role of Th17 cells in *H. pylori* infection has not been clear till now, although a few reports link the IL-23/IL-17 cytokine axis to infection-induced gastritis[49]. IL-23 is overexpressed in the gastric tissue of *H. pylori*-infected patients. Blocking IL-23 in cultured lamina propria leucocytes from infected patients leads to decreased IL-17 production. Furthermore, the treatment of *H. pylori*-infected mice with anti IL-17 antibody led to a more pronounced Th1 phenotype and increased gastritis[50]. The conclusion that IL-17 has a less inflammatory effect on the *H. pylori* infection was indirectly confirmed by the observation that IL17-/- mice showed a reduced colonization after infection, with a concomitant decrease of neutrophil infiltration[51]. This could indicate that IL-17/Th17 induces more inflammation, whereas Th1 leads to increased protection. A different result was observed by blocking the IL-17 signaling pathway. Algood *et al*[52] infected IL-17RA-deficient mice (IL17RA-/-) with *H. pylori*. Although one month after infection no differences were observed between knock-out and wild-type mice, eight weeks later IL17RA-/- animals showed higher colonization. This was accompanied by enhanced *H. pylori* serum antibody levels, more inflammation and an increase in B-cells and plasma cells in the gastric tissue. Like in IL-17-/- mice, the receptor-deficient mice had decreased neutrophil infiltration. Furthermore, elevated levels of IL-17 and IL-21 were observed in IL17RA-/- animals[52]. This implicates that IL-17 positively acts on neutrophil recruitment, whereas IL-17 signaling on B-cells regulates their attraction to the gastric tissue by a negative feedback loop.

On the other hand, in vaccination settings, the induction of IL-17 secretion seems to directly correlate with decreased *H. pylori* colonization. Mice immunized with *H. pylori* lysate showed an induction of Th17 cells and gastric infiltration of neutrophils, which correlated with protection[53]. In addition, the group of Michetti recently showed in a *H. felis* model that vaccine-induced IL-17 production reduced bacterial colonization, indicated by increased infiltration of IL-17-producing CD4+ T-cells (Th17), whereas anti IL-17 treatment inhibited reduction of *Helicobacter* as described before[54]. Nevertheless, it is still possible that the observed vaccine-induced grade of protection is due

to an increased IL-17 secretion according to higher levels of Th17 cells. Taken together, the distinct role of Th17 cells and/or IL-17 in *H. pylori* infection needs to be further clarified with better defined mouse models in which Th17 cells can be selectively manipulated.

Treg

Besides the reported immune-modulatory effect of *H. pylori* on effector T-cells, it has also been described that the pathogen can induce immune-suppressive mechanisms, such as the induction of Treg. Several papers show a correlation of *H. pylori* infection and an increase of Treg in human gastric tissue[55-57]. This effect was much more pronounced in tumor compared to tumor-free gastric mucosa[58]. The severity of gastritis seems to be accompanied by increased numbers of Treg in the inflamed gastric mucosa[55], which is also observed in mouse models of *H. pylori* infection[56]. Additionally, Rad *et al*[56] could show that depletion of Treg led to a more severe gastritis as reflected by an increase in gastric macrophages, T- and B-cells, and a reduction of *H. pylori* colonization. The *H. pylori*-induced inflammation seems to be suppressed by IL-10 producing Treg, because IL-10 deficient Treg are less efficient in the suppression of gastritis[59]. The accumulation of Treg in *H. pylori*-infected gastric tissue is also especially pronounced in children, presumably due to the preference of the early immune system to induce tolerance by the induction of peripheral Treg. The group of Anne Müller could show that neonatal infection in mice with a cagPAI proficient *H. pylori* strain induces tolerance to the pathogen, depicted by higher colonization and less pathology[60]. In context with the tightly regulated gut immunity, Treg seem to support *H. pylori* colonization and the development of a chronic state of infection by suppressing protective immune responses.

VACCINATION AGAINST *H. PYLORI*

Over the last decades many experimental approaches to mediate protection against *H. pylori* infection have been carried out. Thereby, different vaccine formulations with different antigens, adjuvants and application routes have been tested. Several protocols led to significant bacterial reduction in prophylactic as well as therapeutic approaches; however, they almost never reached sterilizing immunity. The most promising vaccine-induced immune reaction seems to be achieved by mucosal priming and a systemic boost[61].

Studies in mice

In classical immunization protocols *H. pylori* lysates or several *H. pylori* proteins in different combinations were used, showing a certain level of protection. Promising antigens were urease, katalase, VacA, CagA, NapA, HpaA, AlpA and BabA. These protocols followed different routes such as oral, intranasal, rectal, intraperitoneal, intramuscular and subcutaneous, involving different adjuvants, like cholera toxin (CT), CpG-oligonucleotides, heat-labile enterotoxin (LT), Alum or Freund's adjuvants. Combinations of more than one antigen often showed better protection[62,63]. In recent years several new vaccine approaches have been introduced. These are comprised of new antigens, new antigen combinations or new adjuvants.

A new vaccine candidate is the 20 kD outer membrane lipoprotein Lpp20[64]. It has been shown to protect mice from *H. pylori* infection by passive anti-Lpp20 antibody transfusion[65]. Li *et al*[64] mapped two MHC Ⅱ-restricted peptide antigens of Lpp20 that induced similar proliferative T-cell responses as the recombinant protein and showed an additive effect when used in combination. If this antigen will generate successful protection, data has to be evaluated.

AhpC (alkyl hydroperoxide reductase) was recently tested as a novel subunit vaccine[66]. It is described as an essential, immunogenic antioxidant protein of *H. pylori* that protects the bacteria from oxidative stress. This study investigated the prophylactic efficacy of AhpC for mucosal (oral) and systemic (subcutaneous) application. Mucosal administration with CT significantly reduced bacterial colonization. Although not significant, systemic vaccination with Alum led to sterilizing immunity in 50% of the animals. A similar picture was observed for the therapeutic efficacy of systemically administered AhpC (over 60% protection). Furthermore, O'Riordan *et al*[66] tested mannosylated AhpC (mAhpC) in both approaches by subcutaneous application with Alum. mAhpC was generated through expression in yeast instead of *E. coli*. In the prophylactic setting, sterilizing immunity was observed in over 70% of the animals and almost 50% in the therapeutic setting. These data exhibit very promising results regarding efficacy. Another important observation is that AhpC or mAhpC alone (without adjuvant) has an almost similar effect without showing strong humoral immunity. What drives the protective effect of the non-adjuvanted protein has to be further investigated.

Additional antioxidant proteins from *H. pylori* were tested as single- and multi-component vaccines[67]. In this prophylactic approach mice were immunized with recombinant superoxide dismutase (SOD), catalase (KatA) and/or thiolperoxidase (Tpx) comparing systemic (subcutaneous) *vs* mucosal (intranasal) immunization. Both routes induced significant reduction in colonization for the single antigens as well as for their combination (Tri-Vac). Interestingly, there was no additive effect in the Tri-Vac group. Further important information from the study is that the mucosal immunization with Tri-Vac and CT induced lower levels of antibody responses compared to the single antigens. This was not observed in the systemic approach formulated with ISCOMATRIX. If the differential antibody induction is due to the route of administration or the different adjuvant used, this could be an important issue regarding recombinant, multi-component vaccines and a potential loss of efficacy.

Another concept to apply a multi-component vaccine is protein fusion. The combination of the outer membrane proteins Omp22 and HpaA was tested in a

prophylactic setting[68]. Mice were immunized with single antigens, their combination and the fusion protein by oral administration. Mutant LT served as adjuvant. A significant protection was achieved in all groups, which was more pronounced with the protein combination or fusion. Immunological parameters like specific antibodies or T-cells were not addressed and thus, it is not possible to make any correlations between the immune response and efficacy. Nevertheless, these data indicate that the combination of antigens can be beneficial.

Another interesting approach uses attenuated *Salmonella* and poliovirus, which express *H. pylori* antigens, as vector delivery systems. The tested *Salmonella* strains expressing urease A and B mediated a significant degree of protection through prophylactic intranasal[69] and oral administration[70]. Also, a poliovirus-based vaccination using urease B as antigen displayed prophylactic, as well as therapeutic efficacy[71]. More recent data combined the *Salmonella* vector approach with a new antigen[72] and a fusion construct of three antigens[73]. *Salmonella*-delivered outer inflammatory protein A (OipA) was used for oral therapeutic immunization[72] and compared to a codon-optimized construct that expresses around 6-fold higher protein amounts. Vaccination induced significantly higher levels of OipA-specific antibodies as well as specific T-cells which had a mixed Th1/Th2 phenotype (IFNγ/IL-4). Furthermore, the adaptive response was significantly higher when mice were vaccinated with the optimized construct. This indicates that the increased amount of OipA produced by *Salmonella* is able to boost the immune response. Vaccination also reduced the colonization with *H. pylori* significantly and was more effective with the optimized vector. The other therapeutic *Salmonella*-based approach included CagA, VacA and UreB in the vector[73]. Liu *et al*[73] compared different constructs where the antigens were combined in all possible orders. Interestingly, *Salmonella* expressing CagA-VacA-UreB (CVU) showed the most drastic effect on colonization with a clearance rate of more than 60%. The other constructs had no or only moderate effects. CVU also developed the highest antibody (IgG and mucosal IgA) and Th1 T-cell response. Unfortunately the immunological assays were only performed with *H. pylori* lysate. Thus, it is not possible to draw any conclusion on differential induction of vaccine-specific immune responses and efficacy. Overall, optimizing the *Salmonella* approach by antigen selection and or codon-optimization seems to be a successful strategy, at least in animal models.

Relatively new experimental vaccine candidates are multi-epitope approaches. Li *et al*[74] used three T-cell epitopes of urease B, and two B-cell epitopes from urease B and HpaA that were generated by software prediction, allowing the induction of a cellular as well as a humoral immune response. The antigens were generated as a peptide fusion protein that was linked to the adjuvant LT beta. In therapeutically immunized mice, the specificity of the three T-cell epitopes clearly could be shown in peptide restimulation experiments, whereas the induction of specific antibodies was tested with *H. pylori* lysates. Nevertheless, oral immunization of already infected mice led to the induction of vaccine-specific CD4+ T-cells and *H. pylori*-specific serum antibodies that induced a clear reduction in bacterial load. Another approach, named Epivac, used a fusion protein comprised of predicted CD4+ T-cell epitopes from HpaA, UreB and CagA[75]. In a prophylactic vaccination setting, mice were immunized subcutaneously in combination with different Th1 promoting adjuvants (CpG, MDP, MPLA and Addavax). Four weeks post-infection a significant reduction in colonization could be observed in all vaccination groups. Although adjuvanted Epivac immunization exhibits a more pronounced Th1 response (IFNγ), the addition of the different adjuvants had only a minor effect compared to the multi-peptide antigen alone. All formulations induced Epivac-specific serum responses, but no IgA in stomach mucosa. The failure of the different adjuvants regarding protection remains an open question. Perhaps they are not capable of inducing a mucosal immune response, reflected by the lack of stomach IgA. Moss *et al*[2] introduced a new peptide-based concept of an *in silico*-based vaccination approach. Conserved and potential immunogenic CD4+ T-cell epitopes were screened by bioinformatic algorithms and further selected *in vitro* and *in vivo*[76]. This gene-to-vaccine approach included multiple epitopes from different antigens in a DNA-prime/peptide boost vaccine. Therapeutic intranasal application induced a broad immune response measured by IFNγ and a significant reduction in colonization compared to intramuscular application or immunization with *H. pylori* lysate. This unbiased genome-based approach may indicate that there are a substantial number of potentially protective antigens, and that the combination of different antigens could be a promising strategy.

Besides the choice of antigen and their combinations, the employment of mucosally active vaccination strategies seems to play a major role in *H. pylori* vaccine efficacy. Regarding clinical use, it is impossible to transfer strong mucosal adjuvants like CT or LT to humans because of their toxicity. The flagellin of *H. pylori* (FlaA) evades recognition of TLR5[77,78]. To facilitate this molecule as a mucosal adjuvant, Mori *et al*[79] constructed a chimeric flagellin (CF) comprised of the hypervariable domain of FlaA and the C- and N-terminal segments of *E. coli* flagellin (FliC) to maintain *H. pylori* specificity and to gain TLR5 activity. CF was shown to activate TLR5 in transfected HEK293 cells. After immunization with or without Alum, a strong, long-lasting (8 mo) IgG serum response could be detected superior to FlaA immunization. Specific IgA could be detected until 3 mo post-immunization for CF + Alum. Furthermore, the immune response after CF + Alum administration shifted to Th1. In a prophylactic immunization study against *H. pylori* CF + Alum administration, given in a combination of intranasal prime/subcutaneous boost, displayed the most significant reduction in colonization compared to CF or FlaA alone or to FlaA + Alum. Although *H. pylori*-specif-

ic T-cell responses were not measured in this paper it can be concluded that the strong induction of specific and also mucosal antibodies by Alum-adjuvanted CF can lead to protection. The application of an adjuvant with antigenic property in combination with potential protective antigens enables new perspectives for future investigations. Nevertheless, the potential toxicity of CF has to be evaluated in appropriate models. Interesting observations have been made regarding *H. pylori* lipopolysaccharide (LPS). Immunization studies with *H. pylori* sonicate indicated an immune stimulatory role of LPS[80]. Lysate that was depleted for LPS induced a reduced Th1 cytokine response (IFNγ, TNFα, IL-2) and an increase in Th2 cytokines (IL-4, IL-5). Therefore, *H. pylori* LPS could serve as an interesting vaccine component. This effect has to be further investigated, but together with an appropriate adjuvant it could aid in protection.

Another focus of the *Helicobacter* field lies in the facilitation of toxin-based adjuvants, as CT and LT provide promising results in experimental vaccination studies. One possibility is to detoxify the adjuvant and simultaneously maintain the stimulatory effect. In a recent study, a double mutant form of LT (dmLT) was used in a prophylactic *H. pylori* vaccination in comparison to CT[81]. By sublingual and intragastric immunization, both adjuvants induced similar protection with *H. pylori* lysate. Sublingual administration of UreB and HpaA formulated with dmLT or CT also led to comparable protection. Both adjuvants induced a similar response regarding T-cell proliferation, specific cytokine induction (IL-17, TNFα and IFNγ), specific serum IgG and gastric inflammation. Only the production of specific gastric IgA was more pronounced in the CT group. Taken together, dmLT seems to be an attractive, mucosal adjuvant with reduced toxicity and preserved stimulatory capacity. Besides LT-based adjuvants, the utilization of CT-based adjuvants is investigated. The group of Nils Lycke approached the construction of chimeric CT by exchanging the GM1-binding subunit B through two copies of the DD fragment from *Staphylococcus aureus* protein A[82]. This so-called CTA1-DD combines the activity of the holotoxin (CTA1) and an immunoglobulin binding domain (DD fragment) that targets and activates mainly B-cells. This adjuvant shows no toxicity in rodents[83] as well as in non-human primates[84]. Significant reduction of *H. pylori* colonization was observed when administered intranasally with lysate in a therapeutic setting[85]. CTA1-DD induced specific IgG, CD4+ T-cell infiltration and a Th1 dominated T-cell response. Compared to CT, the overall effect was less pronounced. Additionally, CTA1-DD induced less gastric inflammation than CT and no specific IgA in gastric mucosa. This can be explained by the targeting and thereby reduced binding capacity of CTA1-DD compared to CT. Overall, the features of this adjuvant make it an interesting candidate for future development.

Studies in humans

In humans several clinical studies were carried out to test the safety and immunogenicity of different vaccine formulations[1]. Mainly, all of these approaches used recombinant urease as the antigen. The oral immunization of asymptomatic *H. pylori*-infected patients was well tolerated, but no specific immune response was induced[86]. By the addition of LT as adjuvant, specific antibody production could be detected[87], concomitant with a reduction of *H. pylori* colonization in infected patients[4]. However, the toxicity of LT led to severe diarrhea. By limiting the amount of adjuvant in the vaccine these side effects could be overcome, but then also a specific immune response was undetectable. To circumvent the problem of toxicity, urease and LT were administered rectally but only a weak immune response was induced[88]. Another vaccine formulation employed killed whole *H. pylori* and a mutant form of LT with diminished toxicity[89,90]. Oral administration exhibited secretion of specific IgA in salivary and feces, but already infected patients did not eradicate *H. pylori* to any degree. Furthermore, urease-expressing *Salmonella*-based delivery vectors were tested in human studies[91-93]. Urease-specific immune reactions were undetectable or only at very low levels after oral vaccination of uninfected volunteers. This is an impressive difference to the findings observed in mouse model experiments. A recent study combined different promising antigens, CagA, VacA and NapA, which seem to play important roles in the severity of *H. pylori* infection[94]. The vaccine was formulated with the very well established adjuvant Alum and administered intramuscularly. Both route and formulation seemed promising because their application is already established in approved vaccines. *H. pylori*-negative volunteers were immunized and no side effects were observed. This vaccine induced specific antibody production against all three antigens and an increased cellular immune response by IFNγ secretion. The same group applied this vaccination strategy in a phase Ⅱ trial in experimentally *H. pylori*-infected healthy volunteers (unpublished data)[95]. Although immunogenicity was achieved, no statistical difference between the placebo and the vaccine group could be detected regarding protection from *H. pylori* colonization. This may be due to the fact that the experimental infection only worked in around 50% of participants whereas the other half cleared the infection. Whether this vaccination will give rise to protection against *H. pylori* infection has to be reconsidered. Therapeutic immunization of naturally infected patients could be an alternative setting to test this trivalent vaccine in future.

CONSEQUENCES FOR VACCINATION IN THE FUTURE

Since *H. pylori* coevolved with humans within the last 88-200000 years[96], it is well adapted to the gastric physiology. Furthermore, the epidemiological and experimental data on its beneficial role in asthma disease[97,98] might lead to the conclusion that *H. pylori* has a commensal-like nature. Although the pathogen induces an overall regula-

tory immune phenotype by regulatory T-cells and tolerogenic DCs, it still can induce strong inflammation and eventually even regulate the degree of inflammation[99]. Perhaps *H. pylori* benefits from this milieu which provides nutrition that enables its survival. On the other hand, experimental animal models indicate that an increase in inflammation leads to subsequent reduction in colonization[56]. As mentioned before, *H. pylori* also induces a pronounced but non-protective T- and B-cell response but at the same time is able to evade the immune response facilitated by several virulence factors[100]. For vaccination these observations are of potential interest. Breaking tolerance and increasing inflammation in combination with essential bacterial antigens could be the important issues a successful vaccine should address. Indeed, experimental immunizations in animal models using strong adjuvants, induction of mucosal immunity and conserved antigens exhibited a certain degree of protection. Still, until now all human vaccination trials that showed immunogenicity have never led to vaccine-induced clearance in *H. pylori* colonization of the stomach. Only one study, working with LT as adjuvant[4], showed a protective effect, but toxic side effects exclude broad application of this vaccine formulation for *H. pylori* immunization. The promising triple antigen vaccine (CagA, VacA and NapA)[94,95] has to be further evaluated by meaningful therapeutic approaches.

Taken together, this is in strong contrast to the experiences achieved from the experimental mouse model. To really generate and improve the efficacy of human *H. pylori* vaccines, mainly two questions have to be solved: what is the right antigen and what is the best adjuvant applicable? On the adjuvant side, the range of choice is limited. All promising mucosal adjuvants used in experimental animal models are not approved for humans and no approved adjuvant is assigned for mucosal administration. There are some efforts to utilize the less toxic cholera toxin subunit B (CTB) for vaccinations[101], but until now it is unclear if this will lead to a human approach. Additionally, it has to be solved whether CT or CTB can efficiently induce protection, as some papers reported an inhibiting effect of CT on the induction of a Th1 response[102,103], the supposed protection mediating arm of immunity in *H. pylori* infection. Some of the recently used adjuvants tested in the mouse model of *H. pylori* infection, like chimeric flagellin[79] or double mutant LT[81], show promising results. Nevertheless, their development towards clinical application is missing. The flagellin approach is still under experimental evaluation. Preliminary toxicity data are still missing. The dmLT was investigated more extensively. The adjuvant did not result in increased intestinal weight in an enterotoxicity assay in mice[104]. Recently, the dmLT was tested in a preclinical mouse model[105] to evaluate the improved immunogenicity of a previously tested vaccine against enterotoxigenic *E. coli* that failed to induce efficacy in a phase Ⅲ clinical trial[106]. The other toxin-based adjuvant, CTA1-DD, has also been tested in different toxicity assays and proven to be save in mice[82]. It was reported to be well tolerated in cynomolgus macaques[83] and rhesus macaques (own unpublished data). The development of adjuvants towards clinical use is not easy to implement. Production under GMP and toxicity testing under good laboratory practice (GLP) is cost extensive and thereby often has to be transferred from a scientific environment to a commercial utilization. The high risk of failure implemented in these developmental steps reduces the dedication of potent companies. Despite all difficulties that have to be faced, new mucosal adjuvants that can be applied in humans are of great interest.

On the other hand, the role of the right antigen remains an open question. Although a lot of different compositions with dead *H. pylori*, whole lysate, single antigens or antigen mixtures have been evaluated, proof of efficacy in humans is missing. New promising candidates like AhpC[66] or OipA[72] have been tested in mice and vector-based approaches and/or multicomponent vaccines have been investigated. In our opinion the right antigen has to be an indispensable virulence factor to circumvent the evasion mechanisms of *H. pylori*. In this context, a potentially protective antibody response through B-cells could be of special importance. An antibody-mediated neutralization of such a factor will disarm *H. pylori* and liberate the immune system to eliminate the pathogen. The gamma-glutamyl-transpeptidase of *H. pylori* could have this potential as it seems to be expressed in most of the clinical isolates (unpublished data) and it inhibits T-cell proliferation, thus blocking the most important defense mechanism in *H. pylori* immunity. Indicated by the work of Moss *et al*[2], a diverse mixture of highly conserved antigens could further improve a successful vaccine. However, we obviously have not been able to translate successful experiments from rodents to the human system until now. It seems that regardless of adjuvant or antigen used, vaccination in mice often exhibits efficacy to a certain extent. Therefore, it is questionable if the mouse is the optimal preclinical model. Lessons we perhaps can learn when working in rodents include the understanding of the mode of action of our vaccine approach. Do we induce a functional B-cell response that can neutralize certain bacterial functions? Do we induce a local B- and T-cell response? What is the exact phenotype of the induced cells and what are the differences to the human system? Perhaps by a more careful investigation of our vaccination models in terms of immunity we will improve clinical outcome in future.

CONCLUSION

Although *H. pylori* may have a beneficial role in asthma and allergic diseases and the prevalence of infection in developing countries is decreasing, an effective vaccine against *H. pylori* is still necessary in the light of the enormous socioeconomic costs associated with this infection. The rising resistance rates of current antibiotic-based therapies require novel therapeutic approaches. Addition-

ally, the high prevalence of *H. pylori* infection in East Asian countries or India requires effective treatment. Furthermore, the prevalence of gastric cancer development is increased in these countries compared to the western world. Antibiotics will probably not achieve mass eradication. Only efficient vaccination would be able to solve these problems and prevent gastric cancer on a population-based level. Until now, a clear path towards protection has been missing. Every year, promising approaches come on the scene. We have to communicate that especially in *H. pylori* vaccination in rodent models, efficacy alone is not sufficient for the clinical outcome. Perhaps we have to investigate the vaccine activity in more detail to convince potential sponsors to invest in future development.

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