Vaccination against *Helicobacter pylori* – A review

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CERTIFICATE

This is to certify that Sadia Rehnuma Ferdous has completed the thesis entitled **"Vaccination against *Helicobacter pylori* – A review"** as a fulfillment of the requirements for the degree of Bachelors of Science in Microbiology thesis part by the BRAC University Dhaka, Bangladesh. Her work is original and the work is up to our full satisfaction.

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Abstract

Since the colonization of H. pylori has sweeping wellbeing results, it speaks to a huge general wellbeing challenge. Current medicines utilize numerous anti-infection agents in blend with corrosive concealment pharmaceuticals to annihilate it. Because of an expansion in anti-microbial obstruction, it is currently harder to dispose of, and the improvement of an antibody as an elective treatment is of expanded intrigue.

In this way, H. pylori is the significant reason for gastric malignancy around the world. It has been named a class i cancer-causing agent by the World Health Organization. Since its revelation in the mid-eighties by Warren and Marshall, inquire about has been centered on the examination of H. pylori science, have pathogen communication, anticipation and treatment. Despite the fact that H. pylori incites a solid humoral and nearby cell invulnerable reaction, the pathogen isn't cleared and sets up a ceaseless contamination after experiences in adolescence. The capacity to colonize the stomach is interceded by a few harmfulness factors that change the host condition, elevate grip to the epithelium, impact the gastric aggravation and prompt safe avoidance. H. pylori can be annihilated by anti-toxin treatment in mix with a proton-pump inhibitor, yet viability is diminishing. Current treatments are costly, have symptoms and add to expanding anti-toxin opposition, underlining the requirement for novel therapeutics. That is the reason immunization advancement against H. pylori remains a focal point of research. Advancement is made yet is incremental. There is requirement for a still better comprehension of the defensive instrument and for enhancing adequacy, sooner rather than later.

Contents

Introduction

Foundation of Helicobacter pylori contamination as an etiologic specialist of peptic ulcer ailment and other gastric pathologies denoted a transformation in gastroenterology which impelled a huge enthusiasm for gastric physiology and immunology explore.

Helicobacter pylori (H. pylori) is a gram negative, winding, pathogenic, extracellular bacterium that colonizes the stomach. It unequivocally cooperates with the gastric epithelium and for the most part causes asymptomatic gastritis. The colonization of H. pylori prompts ulcer advancement in around 20% of contaminated patients and may advance to gastric growth or mucosaassociated lymphoid tissue lymphoma in 1%. In this way, H. pylori is the significant reason for gastric tumor around the world. It has been named a class i cancer-causing agent by the World Health Organization. Since its disclosure in the mid-eighties by Warren and Marshall, explore has been centered on the examination of H. pylori science, have pathogen communication, counteractive action and treatment. In spite of the fact that H. pylori prompts a solid humoral and nearby cell insusceptible reaction, the pathogen isn't cleared and builds up a constant disease after experiences in youth. The capacity to colonize the stomach is intervened by a few harmfulness factors that change the host condition, elevate grip to the epithelium, impact the gastric aggravation and instigate invulnerable avoidance. Around half of the aggregate people is sullied with Helicobacter pylori microorganisms in the stomach. While a great many people remain asymptomatic, 10 to 15% make appearances, for instance, dyspepsia and peptic ulcers, and steady ailment with H. pylori has been recognized as a strong peril factor for the change of gastric adenocarcinoma. (WHO, 1994)

In the previous decade, a few antibody hopefuls against H. pylori have been assessed in creature models. We and others have demonstrated that, other than particular H. pylori antigens, a powerful adjuvant is expected to prompt assurance against H. pylori disease after mucosal inoculation. In this way, vaccination with entire cell or lysate arrangements of H. pylori together with adjuvants, for example, cholera poison (CT) or warmth labile poison (LT) and at times additionally mutant types of the poisons presents insurance against H. pylori disease. CT, regularly utilized in the preclinical assessment of mucosal applicant immunizations, advances solid T cell and also B cell reactions to antibody parts and is a brilliant standard for testing elective mucosal adjuvants. CT is enterotoxic in people, causing abundant looseness of the bowels and liquid misfortune, making it imperative to locate an option, nontoxic mucosal adjuvant that could advance a solid defensive insusceptible reaction against H. pylori contamination. Clinical preliminaries of hopeful H. pylori antibodies have been performed in human volunteers, however so far there has been restricted accomplishment with respect to assurance instigated against H. pylori disease. Albeit upgraded resistant reactions to antibody parts were accounted for in a few investigations, the watched unfriendly impacts of the adjuvants utilized have ruined the further advancement to clinical preliminaries.
A noteworthy concentration in mucosal adjuvant research for quite a while has been the age of nontoxic subordinates of CT or LT that still hold huge adjuvanticity. (Sutton, 2013).

The whereabouts of Helicobacter pylori

Morphology:

H. pylori is a helix-shaped (classified as a curved rod, not spirochaete) Gram-negative bacterium about 3 μm long with a diameter of about 0.5μm. *H. pylori* can be demonstrated in tissue by Gram stain, Giemsa stain, haematoxylin–eosin stain, Warthin–Starry silver stain, acridine orange stain, and phase-contrast microscopy. It is capable of forming biofilms (Stark, 1999) and can convert from spiral to a possibly viable but nonculturable coccoid form. (Chan, 1994)

H. pylori has four to six flagella at the same location; all gastric and enterohepatic *Helicobacter* species are highly motile owing to flagella.(Josenhans, 2000) The characteristic sheathed flagellar filaments of *Helicobacter* are composed of two copolymerized flagellins, FlaA and FlaB. (Rust, 2008)

Physiology:

H. pylori is microaerophilic—that is, it requires oxygen, but at lower concentration than in the atmosphere. It contains a hydrogenase that can produce energy by oxidizing molecular hydrogen (H_2) made by intestinal bacteria.(Olson, 2002) It produces oxidase, catalase, and urease.

H. pylori possesses five major outer membrane protein families.[16] The largest family includes known and putative adhesins. The other four families are porins, iron transporters, flagellum-associated proteins, and proteins of unknown function. Like other typical Gram-negative bacteria, the outer membrane of *H. pylori* consists of phospholipids and lipopolysaccharide (LPS). The O antigen of LPS may be fucosylated and mimic Lewis blood group antigens found on the gastric epithelium.[16] The outer membrane also contains cholesterol glucosides, which are present in few other bacteria.[16]

Enzyme maturation, including urease and hydrogenase, is important for *H. pylori* stomach survival. HypA promotes maturation of hydrogenase, in a similar manner to urease maturation which utilizes nickel-binding sites. The *hyp*A mutant showed comparable survival under acidic conditions, suggesting the *hyp*A is not required during acid stress. Urease maturation itself may also be facilitated by UreG via GTP-dependent conformational changes.

Functional studies of a new candidate, iron-sulfur maturation factor, *nfu,* revealed that this mutant has growth deficiency, sensitivity to oxidative stress, lower aconitase activity, higher hydrogenase activity and is unable to colonize the mouse stomach. As the Fe–S cluster maturation may involve more than one protein, protein interaction observation showed that NfU may interact with 15 of the 36 other putative Fe–S containing target proteins.

Genome:

H. pylori consists of a large diversity of strains, and hundreds of genomes have been completely sequenced. The genome of the strain "26695" consists of about 1.7 million base pairs, with some 1,576 genes. The pan-genome, that is a combined set of 30 sequenced strains, encodes 2,239 protein families (orthologous groups, OGs). Among them, 1248 OGs are conserved in all the 30 strains, and represent the *universal core*. The remaining 991 OGs correspond to the *accessory genome* in which 277 OGs are unique (i.e., OGs present in only one strain).

Transcriptome:

In 2010, Sharma *et al.* presented a comprehensive analysis of transcription at single-nucleotide resolution by differential RNA-seq that confirmed the known acid induction of major virulence loci, such as the urease (ure) operon or the cag pathogenicity island. (Sharma, 2010) More importantly, this study identified a total of 1,907 transcriptional start sites, 337 primary operons, and 126 additional suboperons, and 66 monocistrons. Until 2010, only about 55 transcriptional start sites (TSSs) were known in this species. Notably, 27% of the primary TSSs are also antisense TSSs, indicating that—similar to *E. coli*—antisense transcription occurs across the entire *H. pylori* genome. At least one antisense TSS is associated with about 46% of all open reading frames, including many housekeeping genes.(Sharma, 2010) Most (about 50%) of the 5' UTRs are 20–40 nucleotides (nt) in length and support the AAGGag motif located about 6 nt (median distance) upstream of start codons as the consensus Shine–Dalgarno sequence in *H. pylori*.(Sharma, 2010)

H. pylori & gastric diseases

The association between H. pylori and gastric diseases was not readily accepted by the medical community. Spiral bacteria had been described in human gastric tissue biopsies by microscopy as early as 1906 and periodically throughout the next 70 years. (Doenges, 1939) There were studies however that failed to identify bacteria in gastric biopsy specimens. (Palmer, 1954)

Additionally, there was a general acceptance in the medical community that bacteria could not survive in the acidic stomach and that such observations might be artifacts or evidence of bacterial contamination. The cause of gastritis and peptic ulcer disease was predominantly attributed to stress and were treated by neutralizing acid and with surgery.

The debate was renewed in 1983 when Warren and Marshall published their works documenting the presence of spiral bacteria on the gastric epithelium and predominantly associated with active chronic gastritis (Warren, 1983). Importantly, they were also able to culture the bacteria from fresh clinical biopsies with prolonged microaerobic conditions. The bacteria, which would eventually be called H. pylori were termed Campylobacter-like organisms. These two investigators subsequently published a similar study linking the bacteria to peptic ulcers as well (Marshal, 1984). The gastroenterology community however, largely continued to dismiss the link until, in separate studies, Barry Marshall and Arthur Morris fulfilled Koch's postulates by ingesting cultures of H. pylori and demonstrating an associated gastritis and epigastric pain. Barry Marshall and Robin Warren would receive the Nobel Prize in Physiology or Medicine in 2005 for their work which fundamentally changed our understanding of gastric disease and its treatments.

However, Helicobacter pylori Infections are primarily but not exclusively acquired in early childhood and spread through fecal–oral and oral–oral transmission. Infection lasts for the life of the host and while most infected individuals remain asymptomatic, 10–20% develops peptic ulcer disease, 1% develops gastric adenocarcinoma, and < 1% will develop mucosa-associated lymphoid tissue lymphoma. Gastric cancer is the second leading cause of death due to cancer worldwide and large geographic regions including South America, Eastern Europe, and the Far East these levels range from 20 to 40 per 100,000 (Yamoka, 2008).

Prevalance of gastric diseases

The trend of declining prevalence of *H. pylori* infection is continuing, with major evidence available from studies in Europe. However, in some parts of the world, for example, in some countries in the Middle East, the prevalence has remained relatively stable. A number of systematic reviews and meta-analyses have been published during the past year indicating the lowest prevalence rates of the infection in Oceania (24.4%), the highest in Africa (79.1%), and the global annual recurrence rate of *H. pylori* (4.3%). (WHO, 1994) The recurrence rates were found to be directly related to the human development index and prevalence of infection. Several studies have addressed the correlation between *H. pylori* infection and sociodemographic conditions, source of drinking water and dietary factors. A hypothesis on the role of insects and yeasts in transmitting *H. pylori* has been suggested and addressed. *Helicobacter* sp. have been found in flow flies in Brazil. (WHO, 1994)

So far there is no evidence available that *H. pylori* may survive and persist on the outer body of the fly. Diseases associated with H. pylori infection such as peptic ulcer disease and gastric cancer, and even symptomatic gastritis are manifested predominantly in adults. These diseases create a heavy burden on health care systems world-wide because of the prevalence of H. pylori. The US Department of Health and Human Services estimate that in the year 2004, 20 y after the identification of H. pylori, direct costs associated with peptic ulcer disease reached $2.6 billion in the United States alone. (Ruhl, 2008)

Indirect costs associated with loss of work productivity were estimated to be an additional $518 million. Gastric cancer costs were ~$487 million plus an additional $1.4 billion in indirect costs. Although these diseases are primarily observed in adults, a vaccine administered in early childhood would still be the most practical given the early age of H. pylori acquisition. It is also important to note that H. pylori are often present when children are assessed for abdominal pain or dyspepsia. The first reports of a potential association between H. pylori and gastric health in children were all made in 1986. Three independent teams of physicians investigated biopsies for the presence of Campylobacter pyloridis, or Campylobacter-like organisms as H. pylori were termed at the time (Hill, 1986). Each study examined small numbers of patients including Czinn et al. who performed a detailed histologic analysis of gastric antral biopsies on five patients (Czinn, 1986). Endoscopic evaluation revealed small ulcer, antral nodularity, and histologic gastritis. Histologic evaluation revealed diffuse chronic gastritis including well-formed lymphoid follicles in two patients. Higher magnification revealed the presence of spiral bacteria at the epithelium in all five patients.

These studies were followed by a larger scale prospective study by Drumm et al. in which 67 patients undergoing upper endoscopy and biopsy for gastrointestinal symptoms were examined (Drumm, 1987). The study is notable for several reasons. First, there was a high degree of association between H. pylori and unexplained histologic gastritis. Eighteen of the 67 patients were diagnosed with gastritis by histologic examination.

However, eight of those children had gastritis associated with Crohn's disease, eosinophilic gastroenteritis, or were receiving medication with a known association to increased incidence of gastritis. H. pylori were not detected in any of these cases. H. pylori were detected however in 7 of the 10 remaining patients presenting with histologic gastritis. Second, five of the eight patients with unexplained gastritis and positive for H. pylori were diagnosed with duodenal ulcers by endoscopy. Although H. pylori could be identified in the antrum but not the duodenum, duodenal ulcers were not detected in any of the other 62 patients. Finally, as noted above, H. pylori were not present in any of the biopsies from patients with histologic gastritis due to an underlying cause. Importantly, H. pylori were not detected in any of the 49 patients presenting without any evidence of gross or histologic gastritis. This type of control was lacking from many adult studies at the time and provided strong evidence for H. pylori's unique association with gastric disease in children.

VIRULENCE FACTORS

The *cag* pathogenicity island:

There are several new findings about the role of CagA on the pathogenesis of *H. pylori*infection. CagA is reported to govern the outcome of low iron levels in infected individuals by redistributing transferrin receptors from the cell cytosol to the surface, leading to increased intracellular iron levels. (Flores, 2017) CagA has also now been reported to have another novel interaction partner, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon (YWHAE), also called 14-3-3ε, a member of 14-3-3 family protein. This protein is responsible for activation of NF-κB by CagA.14 In addition to YWHAE, CagA also interacts with CagL and CagY C-terminal domain ($CagY^{B10}$) mediated by the ectodomain of α5β1 ($\alpha5\beta1^{E}$).(Koelblen, 2017) In addition to the effect in the infected gastric mucosa by *H. pylori*, expression of CagA in *Drosophila* intestinal stem cells may promote dysbiosis in the gut. Furthermore, the dysbiotic CagA-dependent microbiota may promote proliferation in the *Drosophila* model. (Koelblen, 2017)

A new observation of the *cag* type four secretion system (TFSS), using electron cryotomography in the presence of host cells, showed dense, periplasmic, and cone-shape tubes spanning the bacterial envelope near membrane tubes. (Chang, 2018) The *cag* TFSS showed a remarkably similar structure to the *Legionella pneumophila dot/icm* TFSS, characterized by outer-membrane associated hat; upper and lower ringlike densities surrounding the hat; barrel like γ density at the structure; central stalk; weak, winglike densities, and parallel elongated densities perpendicular to the membrane cytoplasm.(Chang, 2018)

CagY was reported to play an important role modulating the translocation of CagA and NF-κB and therefore influence inflammation during gerbil infection. (Suarez, 2017) During in vivo infection, *cag*Y sequences may be rapidly altered, resulting in altered activity of *cag* TFSS, which could be attenuated or similar to the parental strain. Similar findings were observed in the strains isolated from humans. (Suarez, 2017) In addition to CagY, the protein structure of CagN and CagM was also characterized. CagN is reported as a helical and monomeric protein with a molecular weight of 34 kDa measured by the multi-angle light scattering (MALS) technique. (Bats, 2018) On the other hand, CagM is a dimeric and helical protein exhibiting MALS peaks at

94 and 418 kDa. (Bats, 2018) Protein interaction analysis in vivo showed that the absence of CagN did not influence the other Cag TFSS proteins. CagM may influence the expression of CagN and other Cag TFSS proteins. CagM could act as a guide protein for the transport of outer Cag proteins (ie, CagL, CagI, and CagN) via the general secretion system. CagN is hypothesized to guide different substrates via the Cag core complex, aided by CagM. (Bats, 2018)

New work investigated *cag* TFSS involvement in the pathogenesis of *H. pylori* infection of the endothelial cell line, HUVEC. (Tafreshi, 2018) Similar to observations in epithelial cells, the *cag* TFSS is a significant factor to stimulate proinflammatory responses, including IL-8 and IL-6 of endothelial cells. Close inspection uncovered a role for CagL induction of IL-8-independent from CagA. Interestingly, integrins α5β1 and αvβ3 were dispensable for infection of HUVEC in contradiction to what has been observed in epithelial cells. (Tafreshi, 2018)

Non-Cag virulence factors

In recent years, work has suggested a role for the HtrA protease in the pathogenesis of *H. pylori*. An exciting new study showed a critical role for HtrA in cleaving E-cadherin to disrupt cell-to-cell junctions, which may allow *H. pylori* to migrate to the basolateral side of epithelial cells and interact with the α5β1 receptor for CagA translocation.The *H. pylori*migration and CagA translocation activity were proportional to the expression of HtrA. (Harrer, 2017) In addition, the HtrA activity was affected by the variation on the amino-terminal between H46/D47 and K50/D51. The amino-terminal variations may affect the oligomerization, secretion, and regulatory activities. The overexpression of HtrA did not affect the expression of other virulence proteins, such as VacA and γ-glutamyl-transpeptidase (GGT). (Harrer, 2017)

A structural similarity analysis using a DALI server, a protein structure comparison server,showed that Lpp20 is very similar to the TNF-α inducing protein (Tip-α), with an additional short α-helix at the N-terminal. (Valeisse, 2017) Cell culture experiments showed that Lpp20 promotes migration and proliferation of gastric cancer cells (AGS) by inducing formation of actin-containing filament around the cell surfaces. In addition, Lpp20 downregulated the mRNA coding for the E-cadherin in AGS cells, which may induce epithelial-mesenchymal transition (EMT), a critical process for tumor progression. (Valeisse, 2017)

Analysis of the *H. pylori* TFSS plasticity zone cluster showed that the whole composition of TFSS in association with CagA positivity may induce higher IL-8 during cell line co-culture and

was influenced by adherence and pH of infection. (Silva, 2017) Lastly, a single operon was found to regulate the expression of its component spanning from VirB2 to VirB10. (Silva, 2017)

Colonization-related virulence factors:

Several colonization factors of *H. pylori* have been described in recent years, including urease, GGT, flolitin-like protein (FLOT), and RhpA. A new gene-silencing technique using the modification of the *ure*A promoter with a *tet*-system developed by Debowski et al. shed new insight on the role of urease during chronic infection. (Debowski, 2017) While urease appeared to be less essential after established colonization, chronic infection was restored by a positive selection process that altered the repressor protein, supporting a continual requirement for urease. (Debowski, 2017) This approach may be applied to other virulence factors for understanding their roles under certain conditions, in either in vitro or in vivo studies. Additional initial colonization factors studied included GGT and RhpA, an RNA helicase protein, mutants of which showed colonization attenuation in the animal model. (El Mortaji, 2018) The GGT is an especially important factor in the early colonization by its metabolic activity and then has additional functions as a defensive agent against the host's immune response. GGT activity promotes a preferred recruitment of $CD8^+$ T cells over $CD4^+$ T cells, whereas RhpA more likely influences motility of *H. pylori* and adaptation to colder environments. In addition, RhpA also regulates the expression of RNase J, which may put this protein in the role of central determinant of gene regulation and virulence control. (El Mortaji, 2018)

Flolitin-like protein is described as a membrane raft–associated protein. (Hutton, 2017) Protein analysis revealed that FLOT is located in the membrane. Functional experiments showed that FLOT promotes cholesterol accumulation by *H. pylori* and increases IL-8 induction. Absence of FLOT may reduce the CagA-dependent activity via the disruption of membrane rafts. Indeed, the absence of FLOT reduced the cell scattering output which is mainly attributed to CagA. In vivo experiments showed the absence of FLOT reduced *H. pylori* colonization in the mice model. (Hutton, 2017)

Outer-membrane protein(s):

The outer-membrane phospholipase A (OMPLA) influences *H. pylori* viability under acidic conditions via a 4 Å transmembrane pore which may allow exchange of urea and ammonium to maintain an optimal periplasmic pH. (Vollan HS,2017) The *ompla* mutant also showed early colonization defects. BabA is known to be important in the initial colonization of *H. pylori*, but

was negatively selected after a few days' and up to 20 weeks' infection in both mice and rhesus macaques. (Hansen, 2018) Observed mechanisms of negative selection of *bab*A included phase variation by slipped stranded mispairing and gene conversion with *bab*B, which may directly confer a fitness advantage. In addition, the negative selection of *bab*A was observed even in the absence of host TLR signaling. (Hansen, 2018)

Another *H. pylori* OMP family member which showed intriguing results was OipA. The *oip*A "on" *H. pylori* reached mid-logarithmic phase faster than the *oip*A "off" strains. Interestingly, the *oip*A "off" strains may induce cell apoptosis faster than *oip*A "on" counterparts. This is most likely due to the suppression of host proteins that suppress apoptosis and/or regulation of cell cycle by the *oip*A "off" strains. However, once purified, OipA was administered to human gastric cell lines (AGS and KATO III); OipA was toxic and induced apoptosis through a Bcl-2 family pathway. (Teymournejad, 2017) These different results between purified protein and OipA producing bacteria might be due to involvement of other bacterial factors.

REGULATION OF VIRULENCE

Transcriptional regulators:

De la Cruz et al investigated whether a variety of stressors that *H. pylori* encounters in the stomach alters the expression of transcription factors as a mechanism to reprogram gene expression. (Vannini, 2017) While most transcription factors did not show differential expression regardless of the conditions, there was marked increase in nearly all transcription factors during biofilm growth, and FliM was the most highly induced. As motility is downregulated during biofilm formation, the authors suggested that this points to the importance of FliM in regulating additional cellular processes. Another study attempted to uncouple direct versus indirect transcriptional effects of the nickel-responsive repressor NikR using a short, high pulse of nickel and a combination of RNA-seq and ChiP-seq. (Vannini, 2017) This analysis suggested that some genes previously attributed to the NikR regulon are secondary targets through upregulation of Fur and noncoding RNAs. While the authors generally argued that NikR was not a global regulator, as only a subset of previous targets confirmed that they were able to identify two new OMPs in the NikR regulon. Mutation of *hopV* induced NikR target genes led the authors to suggest that HopV may be an outer-membrane transporter of nickel.

Salt stress is known to alter *H. pylori* gene expression, but the transcriptional regulators responsible have not been clearly identified. Focusing on high salt induction of OMPs, Loh et al showed that upstream sequences of *hopQ* could confer salt-dependent expression to a heterologous gene. (Loh JT, 2018) They ruled out two known component systems involved in acid adaptation, suggesting that another class of transcriptional regulators may be important. Another study suggested that acid adaptation may be influenced by the nucleoid binding protein HU, which is thought to nonspecifically bind DNA. (Alvarez, 2018) Both transcriptional and proteomic analyses suggested that HU regulates neutrophil activating protein A (NapA), although curiously in opposite directions, highlighting the complexity of integrating transcriptional and post-transcriptional networks.

Two studies deepened our understanding of the roles of specific DNA methylases in regulating gene expression by leveraging SMRT PacBio sequencing to identify the target specificity of the N4-cysteine DNA methylase M2.HpyAII40 and the N6-adenosine methylase Mod5. The target sequences for both of these methylases are underrepresented in the *H. pylori* genome. Mutation

of *m2.hpyAII* leads to altered IL-8 induction, LPS, and gastric cell adherence as well as altered expression of *cag*PAI genes and several OMPs. In the case of Mod5, differentially expressed genes were enriched in motility and OMP genes. In addition to showing altered flagellation of the *mod5* mutant, the author showed that a Mod5 recognition site approximately 500-bp upstream of the *fla*A transcriptional start site was necessary for Mod5-dependent expression of a fluorescent protein promoter fusion. Future work will be needed to determine the mechanisms by which methylation at specific sites alters expression of the target genes.

Post-transcriptional regulation:

A global RNA transcript mapping study revealed a number of putative type I toxin-antitoxin (TA) systems. (Sharma, 2010) New work demonstrated rapid killing by the small peptide toxin of these systems, possibly by depleting ribosomal RNAs, and established a fascinating mechanism of post-translational regulation. (Arnion, 2017) The *aapA1* toxin full-length transcript is not competent for translation due to long-range base pairing interactions between the 3′ and 5′ ends. This transcript is processed by an unknown nuclease leading to an altered secondary structure that is translation competent. Under normal conditions, the antisense transcript *iso*A1 is in large excess and through a loop kissing interaction develops a stable duplex with the Shine-Dalgarno (SD) sequence inhibiting translation and targeting the transcript for degradation by RNase III. Most *H. pylori* genomes harbor multiple copies of this TA system, many of which are associated with mobile genetic elements.

The marked genetic variation among *H. pylori* strains results from mutation and recombination. MutS2 protein counters genetic diversification both through initiating repair of 8-oxo-guanine DNA lesions and suppression of Holiday Junction structures during natural transformation induced recombination. New work has established a novel phase variation mechanism via translational coupling to regulate expression of MutS2. (Wang, 2017) Sequence comparison of the Muts2 upstream sequences revealed no SD sequence and an ATG overlapping with the stop codon of the upstream gene. In some strains, expansion of a repeat sequence separated the stop and start codons rendering them MutS2 “off.” In addition, this study defined a novel specific DNA binding domain distinct from the N-terminal nonspecific sliding clamp and between the ATPase/dimerization domain and the Smr nuclease domain, thus expanding a mechanistic understanding of this regulator.

Genomic diversification as a mechanism for phenotypic variation:

Two studies support a role for genetic variation within the infecting population as a strategy to cope with diverse stresses. One study examined genetic changes that accumulated during gerbil infection with and without high salt diet or iron limitation. (Noto, 2018) A second queried genetic changes during challenge after vaccination with a trivalent vaccine containing VacA, CagA, and NapA or placebo. (Nell, 2017) Both studies found enrichment of nonsynonymous changes in *cag*PAI genes and OMPs. Interestingly, nonsense mutations in vaccine target genes were observed in a subset of the vaccination group, which correlated with higher IgG responses although not statistically significant. The gerbil study found recurrent selection for a specific R88H SNP in the Fur iron-responsive transcriptional regulator. Selection for this mutation could be recapitulated by in vitro growth under high salt or low iron conditions. In a collection of 339 human clinical isolates, the R88H allele was statistically enriched in patients with premalignant lesions compared to nonatrophic gastritis patients, suggesting that this allele may contribute to enhanced pathogenicity. While the gerbil study found unique mutations in all isolates analyzed, including those from the same animal, the human study only analyzed a single isolate per individual. The human study observed phase variation in restriction modification systems. The finding of altered methylation patterns of transcription factor mutations suggests that global effects on gene regulation may arise during chronic infection in addition to the gene specific effects.

While neither the gerbil study nor the human challenge study provided evidence for recombination with unrelated *H. pylori* strains, during natural infection natural transformation is thought to be a major driver of genome diversification. The phospholipase D family nuclease NucT was proposed as a possible regulator of natural transformation. A new X-ray structure indicated a narrow substrate-binding groove consistent with a strong biochemical preference for ssDNA and ssRNA over dsDNA.NucT had a higher affinity for ssDNA than related enzymes and showed endonuclease but not exonuclease activity. With its periplasmic localization, NucT may play a role in degrading single strand DNA generated during unwinding of duplex DNA at the inner membrane transformation channel or may function primarily in DNA and RNA degradation for purine salvage.

The treatment of *Helicobacter pylori* infection

It continues to evolve and remains a topical global research interest. Triple therapy has been modified in that it is now recommended to use double-dose (80 mg) proton-pump inhibitor (PPI), quadruple dose (2 g) amoxicillin, and clarithromycin (1 g) for at least 10 days, and preferably 14 days. (Malfertheiner, 2017) The substitution of vonoprazan, a novel potassium-competitive acid blocker that provides reversible acid suppression by preventing K+ from binding to gastric H+/K+-ATPase, for PPIs has shown promising results, however remains to be tested outside Asia. (Ozaki, 2017)

Quadruple therapy is gaining in popularity particularly in areas with increasing resistance to standard triple therapy. (Tursi, 2017) Tailored, culture-based treatment seems a logical choice and has significant success.However, there is an expense and delay involved, which limits its universal use at present.

Levofloxacin remains one of the most favored second-line therapies; however, bismuth, when available, is an increasingly successful option. Sequential therapy remains in use in areas of high resistance, but may prove challenging in terms of compliance, and is no longer recommended. (Gatta, 2018) Three-in-one formulations of bismuth quadruple therapy (BQT) may improve compliance. Probiotics appear to have some effect on *H. pylori* eradication, as their addition likely improves compliance by reducing the side effects of antibiotics. For example, several meta-analyses reported a gain of 10%-14% in the cure rate from the addition of a probiotic to traditional therapy compared to placebo. High-dose acid suppression and treatment duration of a minimum of 10 days offer the best conditions for success with triple therapy. Several studies in the past year have demonstrated the utility of standard triple therapy in low resistance areas (<20% resistance to clarithromycin). (Miftahussurur, 2017) A retrospective study from the New York metropolitan area reported a cumulative eradication rate of 86% with 10-day Omeclamox®-Pak, a clarithromycin-based triple therapy with once daily omeprazole. A study from Syria highlighted insufficient responses to both clarithromycin and levofloxacin-based triple therapies with eradication rates of 35.1% and 29.7%; however, of note, low-dose (20 mg) esomeprazole was given. (Cheha, 2018) Based on this, Syria likely represents an area of high resistance. Metronidazole-based triple therapy has demonstrated efficacy in areas of high clarithromycin resistance (eradication rate with metronidazole 94.3% vs clarithromycin 72.7%. (Adachi, 2017)

Quadruple therapy, comprised of standard triple therapy with the addition of rifaximin (a nonabsorbable antibiotic), had poor results with eradication rates of 61% in Spain. Metronidazole continues to underperform in triple therapy, with eradication rates of 64% in metronidazole-naive patients.High-dose metronidazole does enhance eradication rates however in areas of high metronidazole resistance,demonstrating that in vitro resistance of metronidazole does not always correlate to in vivo failure.

Resistance to the commonly used antibiotics is increasing in certain areas over time, as demonstrated by a retrospective review from the Netherlands which reported increasing resistance rates for clarithromycin (9.8%-18.1%), metronidazole (20.7%-23.2%), and ampicillin (6.3%-10%) over 10 years. (Ruiter, 2017) A 10-year follow-up study from Italy showed suboptimal eradication rates using amoxicillin and metronidazole, which had not changed significantly (73.5% vs 69.2%) in the past decade. (Ribaldone, 2017) A further study from Italy suggested that while resistance to the most frequently used antibiotics had increased, it had likely plateaued over the past number of years. The first systematic review of primary antibiotic resistance in the Asia-Pacific region reported mean resistance rates of 17% for clarithromycin, 18% for levofloxacin, and 44% for metronidazole. There was, however, significant heterogeneity in resistance rates across different countries in the region.

There would appear to be a maximal acid-suppressive effect with PPI bid dosing in triple therapy. Eradication rates with PPI tid dosing compared to PPI bid dosing in standard triple therapy were not improved. Reports of increasing resistance to the components of standard triple therapy have led to the development of alternative treatment regimens and a general shift away from the traditional treatment protocols, particularly in areas with high antibiotic resistance.

The interest in probiotic therapy as an adjunct to eradication therapy has certainly increased over the past year with the number of publications rising significantly. As the effective treatment duration increases, there is a concern regarding antimicrobial side effects. In one recent study, the addition of a probiotic was shown to reduce the frequency of adverse events from 28.2% in the nonprobiotic group to 12.2%. (Jung JH, 2018)

A meta-analysis reported that the addition of a probiotic to bismuth-based quadruple therapy increases the eradication rate by approximately 10%. A systematic review assessed the efficacy of probiotics as monotherapy. While probiotics were found to be superior to placebo, the pooled eradication rate from the 11 included studies was only 14%.One study evaluated the anti-*H. pylori* activity of seven *Lactobacillus delbrueckii*subsp. *bulgaricus* (GLB) strains. The GLB strains were found to produce heat-stable bacteriocin-like inhibitory substances that had a

strong anti-*H. pylori* activity, rendering them valuable probiotics in the control of *H. pylori* infection. *Lactobacillus reuteri* (2×10^8 CFU *L. reuteri* DSM 17938 plus 2×10^8 CFU *L. reuteri* ATCC PTA 6475) 7 times per day or matching placebo plus 20 mg pantoprazole bid for 4 weeks was used for *H. pylori* eradication in a double-blind placebo-controlled randomized 2-site study. The cure rates per protocol were 3/24 (12.5%; 95% CI 2.6%-32%) with *L. reuteri* vs 1/24 (4.1%) with placebo.

To summarize, probiotics are likely to improve compliance and therefore improve eradication rates of triple therapy. However, the potential benefit of adding probiotics to more recent and effective combinations (such as concomitant or BQT regimens) has yet to be proven.

But given that, Bacterial resistance to antibiotics is considered the most important determinant of treatment failure. Monitoring the evolution of antimicrobial resistance to common antibiotics is therefore of special importance for clinicians. The frequency of resistance to antibiotics in Helicobacter pylori isolates is increasing. So a different line of treatment is needed, which is, Vaccination against H. pylori.

Mechanisms of H.pylori vaccination

H. pylori infection can be treated by established antimicrobial therapies. These therapies can vary but in general consist of combination therapies including two antibiotics and a proton pump inhibitor. The most recent consensus recommendation supports quadruple therapy and a course of 14 days. Eradication rates as high as 90% can be achieved in compliant patients. Unfortunately, as these therapies are taken multiple times per day for a minimum of 7–14 days, patient compliance is often poor. They are also often accompanied by side effects including diarrhea and nausea. In addition, it is not fiscally or practically possible to treat one half of the world's population with antimicrobial agents in an effort to cure peptic ulcer disease or prevent gastric cancer. (Uchiyama, 2017)

In the United States, if only 10% of young people are infected, more than one million pediatric patients would be at risk to receive eradication therapy. The cost is prohibitive and it would also lead to the development of antibiotic resistance in both H. pylori and other human pathogens. Therefore, a concerted effort has been made toward vaccine development. It should be noted that chronic H. pylori infection is associated with several significant health benefits, particularly in the West where a steady decline in the prevalence of H. pylori has coincided with increased esophageal pathologies. An inverse correlation has been described between H. pylori infection and Barrett's esophagus Barrett's metaplasia, esophageal adenocarcinoma, and esophageal eosinophilia. A meta-analysis on published studies also suggests that H. pylori may provide protection from Inflammatory Bowel Disease. More significantly with respect to children, an inverse correlation has been described between H. pylori infection and allergic asthma. The protective function of H. pylori against allergic asthma has been confirmed in an experimental mouse model and demonstrated to be due to the overriding effects of H. pylori induced Treg cells which are able to suppress immune responses against unrelated antigens. The decision to apply widespread vaccination against H. pylori must be considered against the potential increase in other chronic diseases, but in populations where gastric cancer is more prevalent and associated with high morbidity and mortality vaccination would provide an overall advantage.

The nature of H. pylori infection has made development of a vaccine technically challenging. Since H. pylori resides at the surface of the gastric epithelium and does not invade the tissue, it is able to avoid many aspects of the host immune response. Although neutrophils can cross the epithelium and form crypt abscesses, host defenses against the motile H. pylori are limited to secreted antibodies and antimicrobial peptides. It was generally accepted by immunologists that systemic immunizations were ineffective at generating resistance to mucosal pathogens. Additionally, vaccines targeting mucosal tissues also induce weak

immunity. The lack of safe and efficacious adjuvants to strengthen mucosal immunogenicity is largely responsible for the paucity of vaccines against venereal diseases and gastrointestinal infections.

One class of mucosal adjuvants is the bacterial exotoxins such as cholera toxin (CT) and E. coli heat labile toxin (LT). The toxins consist of a pentameric ring of B subunits that bind to GM1 gangliosides present on epithelial cells and an A subunit that has enzymatic activity. Upon endocytosis of the toxin, the A subunit activates a G protein which ultimately leads to continuous production of cAMP resulting in the efflux of ions and water. In small doses however, these exotoxins are not only immunogenic but when mixed in solution with an unrelated protein antigen confer potent immunogenicity to that protein when applied by mucosal immunization. This technique had been employed with great success in mice to study host immunity to Sendai virus as a model for human influenza by the laboratory of John Nedrud (Nedrud, 1987).

Steven Czinn, a pediatric gastroenterologist and early investigator of H. pylori pathogenesis collaborated with Nedrud to adopt this immunization strategy to demonstrate oral immunization could be used to generate a host immune response to H. pylori (Czinn, 1987). Mice were given four weekly doses of 1 mg H. pylori whole cell lysate antigen in combination with 10 μg CT adjuvant by oral gavage. They achieved a fivefold increase in anti-H. pylori serum IgA titers and a 16-fold increase in intestinal IgA compared with mice immunized without CT. Significant increases in IgG were also noted. Similar results were achieved when immunizing ferrets with 7 mg H. pylori lysate and 60 μg CT doses. Ferrets were selected because their stomachs become naturally colonized by the closely related species H. mustelae. This was the first demonstration that it was possible to induce significant levels of anti-H. pylori mucosal immune responses through the use of oral immunization combined with experimental exotoxin adjuvants.

These studies were performed prior to the development of a mouse model for H. pylori infection that could utilize immunocompetent mice. However, the use of the cat isolate H. felis was demonstrated to readily infect mice and to induce histologic gastritis similar to that observed in H. pylori infected humans within several weeks of infection (Lee A, 1990). The development of this animal model made it possible to test the new oral vaccine protocol using a Helicobacter challenge model. Mice were orally immunized with weekly doses of H. felis lysate antigen in combination with CT adjuvant and then challenged 7–10 d after the final immunization. Mice were then examined 7 d following challenge and assessed for immunity.

Similar to their prior study, Czinn et al. observed significant increases in serum and mucosal anti-H. felis antibody titers including a fourfold increase in gastric IgA, and an eightfold increase in intestinal IgA. Most importantly, 76% of immunized and challenged mice were determined to be protected from infection whereas only 22% of control mice were bacteria

free. Similar protection was also observed by Chen et al. employing the same mouse model and closely related immunization protocol. These studies, combined with new animal models for H. pylori infection, served as the foundation for over two decades of studies by numerous laboratories to better characterize H. pylori pathogenesis, identify the immune mechanisms that contribute to protective immunity, and to test multiple variations and new strategies for vaccinating against H. pylori. Protecting mice from H. pylori could be accomplished with many candidate protein antigens, by multiple routes of immunization, and with many alternative adjuvants and delivery mechanisms.

These results, however, while highly reproducible among laboratories were not so easily translatable in clinical trials.

Clinical trials

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.

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 prophylactic setting (Huang, 2013). 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 and oral administration[70]. Also, a poliovirus-based vaccination using urease B as antigen displayed prophylactic, as well as therapeutic efficacy. More recent data combined the Salmonella vector approach with a new antigen and a fusion construct of three antigens[73]. Salmonelladelivered outer inflammatory protein A (OipA) was used for oral therapeutic immunization 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. Liu et al 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 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. 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 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. 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. To facilitate this molecule as a mucosal adjuvant, Mori et al 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 postimmunization 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-specific 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. 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. 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 LTbased adjuvants, the utilization of CT-based adjuvants is investigated. The group of Nils Lycke approached the construction of chimeric CT by exchanging the GM1binding subunit B through two copies of the DD fragment from Staphylococcus aureus protein A. 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 as well as in non-human primates. Significant reduction of H. pylori colonization was observed when administered intranasally with lysate in a therapeutic setting. 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. 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. By the addition of LT as adjuvant, specific antibody production could be detected, concomitant with a reduction of H. pylori colonization in infected patients. 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. Another vaccine formulation employed killed whole H. pylori and a mutant form of LT with diminished toxicity. (Sougioultzis, 2002)

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. 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. 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). 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.

Future perspective

Since H. pylori coevolved with humans within the last 88-200000 years (Moodley, 2012), it is well adapted to the gastric physiology. Furthermore, the epidemiological and experimental data on its beneficial role in asthma disease might lead to the conclusion that H. pylori has a commensal-like nature. Although the pathogen induces an overall regulatory immune phenotype by regulatory T-cells and tolerogenic DCs, it still can induce strong inflammation and eventually even regulate the degree of inflammation. 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. 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 (Fischer, 2009).

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, 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) 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, 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, 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 or double mutant LT, 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. Recently, the dmLT

was tested in a preclinical mouse mode 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. The other toxin-based adjuvant, CTA1-DD, has also been tested in different toxicity assays and proven to be save in mice. It was reported to be well tolerated in cynomolgus macaques (Erikson, 2004) 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 or OipA have been tested in mice and vectorbased 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, 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

In summary, vaccine development against H. pylori remains a focus of research. Progress is made but is incremental. There is need for a still better understanding of the protective mechanism and for improving efficacy. It will also be necessary to evaluate gain by protection versus the alleged danger of the same immune mechanism contributing to disease. Further clinical studies may help to avoid blurring this important issue by incongruent animal models. 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. Additionally, 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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