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REVIEW – RECENT ADVANCES IN THE RESEARCH ON INFLAMMATION, IMMUNITY, AND VACCINES RELATED TO *HELICOBACTER PYLORI*

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Abstract – *Helicobacter pylori* can persistently colonize the human gastric mucosa and cause chronic gastritis, peptic ulcers and gastric cancer. These illnesses develop in a small proportion of infected individuals usually in adulthood, decades after the acquisition of infection in childhood. *H. pylori* triggers both the innate and adaptive immune systems, with a Th1/Th17 cells biased and regulatory T cells response, however, *H. pylori* successfully evades the immune system. Insights into *H. pylori*-induced inflammation and immunity are key to a better understanding the pathogenesis of *H. pylori* infection and to designing and evaluating preventive and therapeutic vaccines. This review presents the main advances in *H. pylori*-induced inflammation, immunity, and vaccines between March 2022 and April 2023. Multiple studies characterized the interaction between pattern recognition receptors and Helicobacter sp. such as Toll-like receptors 9, 7 and 8, AIM2, and STING. The interaction with host cells via annexins was also described, as well as with several proinflammatory and proangiogenic factors involved in angiogenesis. Additional studies focused on the role of CD8+ T cells in *H. pylori* infection, others addressed Th22 CD4+ cells and regulatory B cells. Currently, there is no licensed anti-*H. pylori* vaccine. *H. pylori* vaccine studies were all in pre-clinical phases and focused on antigen discovery, construction, and evaluation of new vaccine candidates, showing promising results in terms of immunogenicity and efficacy in mouse models.

Keywords: *Helicobacter pylor*i, Inflammation, Pattern recognition receptors, Vaccines, CD4+ T cells, CD8+ T cells, Regulatory T cells.

INTRODUCTION

Helicobacter pylori colonizes the stomach and can cause peptic ulcers, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma[1]. *H. pylori* infection is acquired in childhood and following an acute phase, it becomes a persistent infection resulting in chronic gastritis[1,2]. *H. pylori* successfully evades the host's immune system, both the innate and adaptive immunity and typically induces a combined CD4 T helper (Th)1 and Th17 response, coupled with regulatory T (Treg) response, that enables infection persistence[3,4]. While most individuals infected with *H. pylori* do not develop the disease, with time, the persistent gastric inflammation induces damage to the gastric mucosa[2]. Chronic *H. pylori*-associated gastritis pro-

gresses to atrophic gastritis, intestinal metaplasia, and gastric cancer[4]. The risk of peptic ulcer disease and gastric cancer is increased in relation to the presence of virulence factors such as the Cytotoxin-associated gene A (CagA) antigen, a pro-inflammatory and oncogenic protein[1,5,6]. The *cag* pathogenicity island (*cag*PAI), plays an important role in the pathogenesis of *H. pylori* infection; it includes genes encoding *cag* type IV secretion system (*cag*T4SS), which is responsible for the translocation of CagA into the gastric epithelial cells[7]. The interaction between *H. pylori* and the host immune system is pivotal for the understanding of the pathogenesis of the infection and vaccine development. In this article, the recent evidence published between March 2022 and April 2023 on *H. pylori*-related inflammation, immunity, and vaccines is reviewed.

INNATE IMMUNITY, INFLAMMATION, AND INTERACTION WITH HOST CELLS

Pattern-recognition receptors (PRRs) that react to conserved microbial motifs are an integral part of innate immunity. PRRs include Toll-like receptors (TLRs), cytosolic DNA sensor/adaptor proteins (e.g., stimulator of interferon genes [STING]), Nucleotide-binding oligomerization domain (NOD)-like receptors (NLR), retinoic acid-inducible gene-I (RIG-I)-like receptors, C-type lectin receptors, and melanoma-2 (AIM2)-like receptors[8]. Several studies[9-14] assessed PRRs in the context of *H. pylori* infection in the last year (Figure 1A).

It was shown that *H. pylori* affects several TLRs, specifically the upregulation of TLR9, which requires *cag*T4SS (Figure 1A). Tang et al[11] demonstrated the upregulation of TLR9 expression in biopsies of patients with gastric cancer and *H. pylori*-gastritis. TLR9 expression

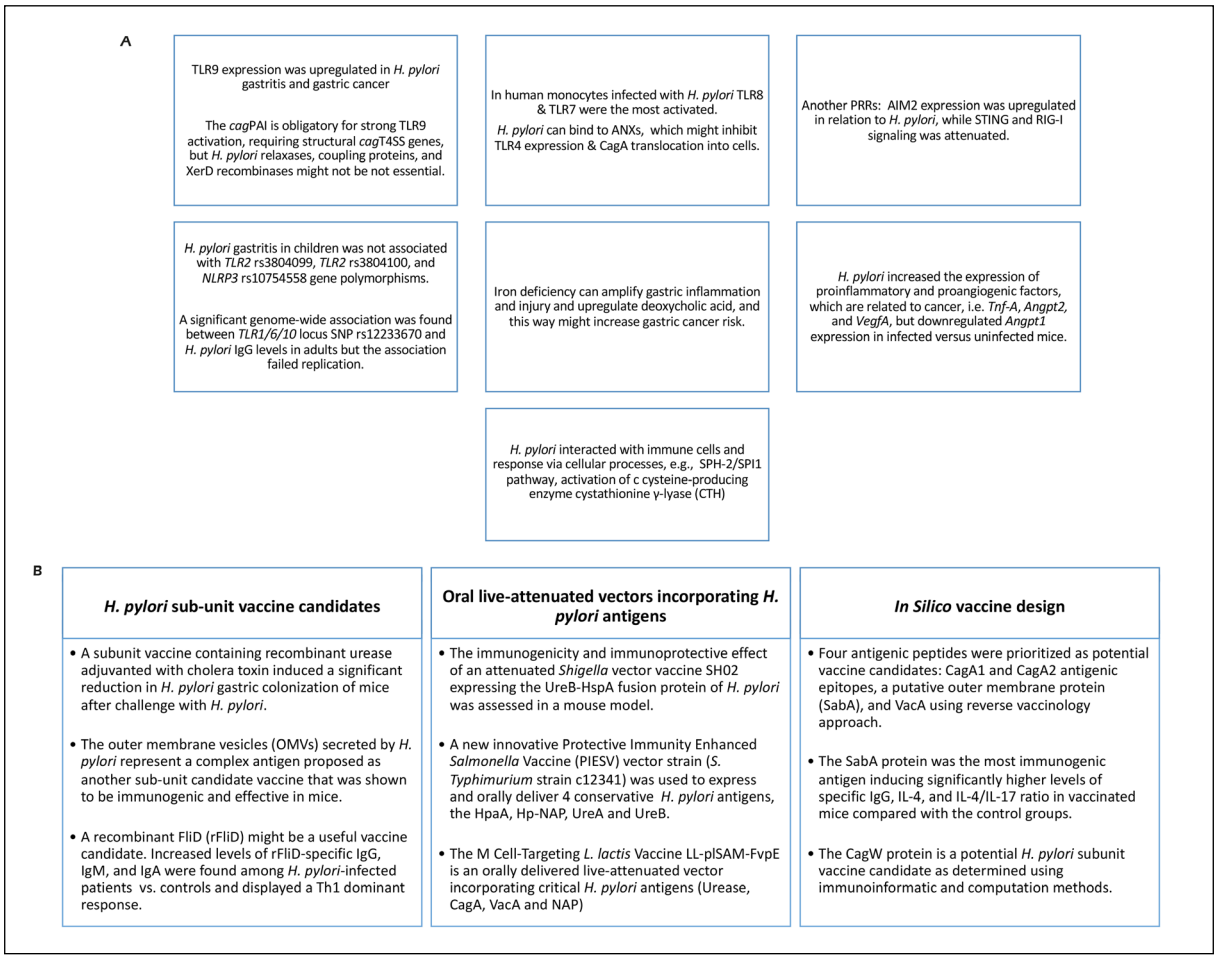

Figure 1. A, Highlights of findings from recent studies that addressed the innate immunity and inflammation in the context of *H. pylori* infection – 2022-2023. **B,** Highlights of recent studies on *H. pylori* vaccine candidates 2022-2023.

was elevated in the gp130$^{F/F}$ mice, a model for inflammation-associated intestinal-type gastric cancer, and chronic *Helicobacter felis* infected wild-type (WT) mice. Results were comparable in experiments with genetic ablation of *Tlr9* in gp130$^{F/F}$ mice and *H. felis* infection in *Tlr9*$^{-/-}$ mice[11] *via* reducing gastric inflammation and cellular proliferation nuclear factor kappa B (NF-κB).

The *cag*T4SS injects CagA and the LPS-metabolite ADP-heptose into epithelial cells, as well as chromosomal bacterial DNA, which activates TLR9, while TFS3 and TFS4 (additional T4SSs on *H. pylori* genome) are likely vital in conjugational DNA transfer[7]. Usually, T4SSs are composed of 11 VirB proteins (VirB1 to VirB11), a DNA processing enzyme (relaxase VirD2), and the coupling protein VirD4/TraG. The VirD2 relaxase enzymes are lacking in the *cag*T4SS, but present in TFS3 and TFS4. Tegtmeyer et al[13] used isogenic knockout mutants of *vir*B9 and *vir*B10 (equivalent to *cag*T4SS structural genes *cag*X and *cag*Y), *vir*D2 (*rlx*1 and *rlx*2), *vir*D4 (*cag*5, *tra*G1/2) and *xer*D recombinase genes in *H. pylori* laboratory strain to assess their involvement in TLR9 activation. The *cag*PAI was obligatory for strong TLR9 activation. The stimulation of TLR9 was attenuated by the inactivation of the structural *cag*T4SS genes *cag*X and *cag*Y (corresponding to virB9 and *vir*B10), but was not affected by the deletion of VirD2, VirD4, or *xer*D genes. Infection with an *xerD2* knockout strain also led to CagA phosphorylation and TLR9 activation during infection. These results were confirmed in human clinical *H. pylori* strains with various T4SSs genes from different regions. Thus, *H. pylori* relaxases, coupling proteins, and XerD recombinases likely are not essential for *H. pylori* TLR9 activation[13].

Lee et al[14] examined the role of TLR7 and TLR8, known sensors of viral single-stranded RNA, in *H. pylori* immunity. Utilizing human THP-1 monocytes infected with *H. pylori* J99 or SS1 strains, they showed that TLR8 was the most upregulated transcript, followed by TLR7. Treatment with TLR7/8 antagonist, annulled *H. pylori* infection-mediated IFN-*α* and IFN-*β* stimulation, and decreased *H. pylori*-related phosphorylation of IRF7[14].

The impact of the immune response on *cag*A copy number was evaluated using a mouse model with different immune statutes. *H. pylori* PMSS1 exhibited an increased *cag*A copy number than those from *Rag1*$^{-/-}$ mice lacking functional T or B cells, but a lower copy number was found in *Il10*$^{-/-}$ mice, showing strong immune response[15] than WT mice. The *cag*A copy number was positively associated with IL-8 release as well as the recombination in *cag*Y (which affects T4SS)[15].

Additional PRRs were examined in relation to *H. pylori* infection. A study[9] that assessed the role of AIM2 in the pathogenesis of *Helicobacter*-related gastric disease, showed that AIM2 mRNA and protein expression were increased in gastric biopsies obtained from *H. pylori*-positive than *H. pylori*-negative patients. The results were strengthened by using chronic *H. felis* infection in Aim2$^{-/-}$ mice, which exhibited reduced severity of gastric inflammation and hyperplasia compared to WT mice, along with reduced proliferation and apoptosis of epithelial and immune cells. These findings negatively correlated with inflammasome activity and IL-1β[9].

Dooyema et al[10] examined the interaction of *H. pylori* with STING. Using *in vitro* and *ex vivo* experiments they showed that *H. pylori* can actively attenuate STING and RIG-I signaling through downregulation of the transcription factor interferon regulatory factor 3 (IRF3) activation. Infection in *Sting* deficient mice resulted in an amplified Th17 inflammatory response and expression of Trim30a, a host immune regulator. These findings shed light on mechanisms by which *H. pylori* might moderate the innate immunity and sustain chronic gastric inflammation and injury[10].

At the population level, Melit et al[12] found no significant differences in *TLR2* rs3804099, *TLR2* rs3804100, and *NLRP3 (*NLR family pyrin domain-containing 3) rs10754558 gene polymorphisms among children with *H. pylori*-gastritis, non-*H. pylori* gastritis and control group, but significant differences in systemic inflammation markers between the groups were found within specific genetic variants[12]. The association between *H. pylori* IgG titers and Toll-like receptor *(TLR1/6/10)* locus on *4p14* was re-evaluated in populations of European ancestry[16], demonstrating a significant genome-wide association with top SNP rs12233670, however, with heterogeneity across populations and the association failed replication. Variation at the *TLR1/6/10* locus altered surface expression of monocytes and neutrophils TLR-1 mediated cytokine production[16].

Interactions of *H. pylori* with host cells were addressed in several studies. Host cell annexins (ANXs) encompass a protein family that can bind to membranes, interact with

bacteria and viruses, and might have a regulatory role in inflammation[17]. Schmidinger et al[18] showed that *H. pylori* is capable of binding to ANXs. Binding studies using purified *H. pylori* LPS and *H. pylori* LPS mutants revealed binding of ANXA5 to lipid A, depending on the lipid A phosphorylation. ANXA5 binding substantially inhibited LPS-mediated TLR4 signaling. The levels ANXA2 and ANXA5 were amplified in the gastric tissue of *H. pylori*-infected humans. ANXA5 binding to *H. pylori* LPS limited CagA translocation without affecting the bacterial binding to host cells. This mechanism is likely important in interfering with immune recognition[18].

Further progress was made in understanding potential pathways that might promote gastric inflammation and injury related to *H. pylori* infection (Figure 1). The proliferation of endothelial cells, an essential component in angiogenesis, requires stimulation by growth factors and inflammatory markers, primarily vascular endothelial growth factor (VEGF) family and angiopoietins[19]. In a mouse model, Malespin-Bendana et al[20] examined the levels of mRNA and protein expression of proinflammatory and proangiogenic factors, i.e., Angiopoietin (Angpt) 1, Angpt2, VegfA, Tnf-α, bacterial colonization, inflammatory response, and gastric lesions[20]. *H. pylori*-positive mice exhibited higher expression of *Tnf-A*, *Angpt2,* and *VegfA* at the mRNA and protein levels, but a downregulated *Angpt1* expression *vs.* uninfected mice[20].

The effects of iron deficiency (ID) on *H. pylori*-gastric inflammation and injury were assessed in WT C57BL/6 mice and INS-GAS mice (genetically predisposed to gastric dysplasia)[21]. ID amplified gastric inflammation and injury during *H. pylori* infection. The incidence of gastric dysplasia increased in infected INS-GAS mice kept on an iron-depleted diet *vs.* an iron-replete diet. The levels of proinflammatory chemokines and cytokines increased following *H. pylori* infection, some of which were further amplified under ID. *H. pylori* altered major metabolic pathways under ID conditions with marked upregulation of a carcinogenic bile acid: deoxycholic acid. Bile acid sequestration diminished gastric inflammation injury. Analysis of data from a cohort of 416,885 individuals showed a significant dose-response decrease in gastric cancer risk in relation to cumulative use of bile acid sequestrant[21].

Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling mediates immune regulation. Programmed death ligand 1 (PD-L1) expressed on gastric epithelium can inhibit the immune system. A proteomics analysis of a cohort of patients with gastric lesions and gastric cancer showed an increase in STAT1 with the progression of premalignant lesions to gastric cancer and was linked to poor prognosis in gastric cancer patients. STAT1 was stimulated in *H. pylori* gastritis and markedly increased along its target gene, PD-L1, in gastric cancer. Phosphorylated STAT1 and PD-L1 correlated with immune infiltration and proliferation in mice[22].

MACROPHAGES, MONOCYTES

The role of macrophages in *H. pylori* infection was assessed in 2 studies[23,24]. *H. pylori* upregulates the expression of the cysteine-producing enzyme cystathionine γ-lyase (CTH) in humans and mice[25]. Latour et al[23] showed that genetic deletion of *Cth* led to reduced gastritis, which correlated with reduced macrophage and T cell stimulation in *H. pylori*-infected tissues, and downregulation of metabolic pathways. *Cth*-deficient macrophages displayed changes in the proteome, reduced NF-κB activation, decreased expression of macrophage activation parameters, and diminished oxidative phosphorylation and glycolysis, thus, suggesting that CTH has a major role in macrophage activation, promoting pathogenic inflammatory response to *H. pylori*[23].

Macrophages require energy to sustain metabolic pathways. In this context, the Src homology-2 domain-containing phosphatase 2 (SHP2), encoded by the gene tyrosine-protein phosphatase nonreceptor type 11 (*PTPN11),* is a ubiquitous tyrosine phosphatase implicated in regulating cancer and immune cell signaling[26]. Li et al[24] examined the differential gene expression in normal gastric antrum, *H. pylori*-negative, and *H. pylori*-positive gastritis tissue of children. They demonstrated significant elevation of PTPN11 in macrophages of *H. pylori*-positive gastritis tissue. M1 (proinflammatory) macrophage-associated molecules were highly expressed in *H. pylori*-positive gastritis tissues compared with normal tissues, while the expression markers

of M2 macrophages (more immunosuppression) were decreased in the former. The expression of proinflammatory cytokines *Il-1β*, *Tnfa*, and *Il-6* was increased in macrophages with *H. pylori* infection compared with LPS-treated macrophages. SPH2 activated the glycolytic function of macrophages infected with *H. pylori* which was significantly enhanced compared with that of macrophages treated with LPS. *SPI1* was significantly increased in *H. pylori*-positive gastritis tissues. Thus, the SHP2/*SPI1* axis could be closely associated with glycolytic dysfunction of macrophages in *H. pylori*-infected pediatric gastritis[24].

Repeated stimulations with *H. pylori* did not increase the inflammatory response in primary human monocytes, but primed monocytes with viable *H. pylori* were hyperresponsive to an *E. coli*-LPS stimulation shortly post-infection[27].

The interaction between *H. pylori* and gastric epithelial cells relies on the activation of NF-κB transcription factors regulating the expression of chemotactic factors. The NF-κB signaling pathway is triggered by the bacterial heptose metabolites, which stimulate the host ALPK1-TIFA axis. Sokolova et al[28] demonstrated that ALPK1-dependent TIFA activation in *H. pylori*-infected gastric epithelial cells was followed by a decline in TIFA levels, and this was impeded by inhibitors of the proteasomal and lysosomal degradation. *H. pylori* promoted the interaction of TIFA with polyubiquitin and optineurin, which are involved in intracellular trafficking to lysosomes[28].

IMMUNITY

Recent studies further characterized the immune response to *H. pylori* infection, including the role of CD+8 T cells[29], CD4+ Th22 and Treg cells[30], gastric microenvironment and immune cell types and Th17 role[31], and Breg cells[32].

Evidence on the role of $CD8^+$ T cells in *H. pylori* infection is limited. Koch et al[29] characterized gastric *H. pylori*-specific $CD8^+$ T-cell mediated immunity in mice and humans. They showed $CD8^+$ T-cells with typical tissue-resident memory (T_{RM}) phenotype, which profoundly infiltrated the gastric mucosa of C57BL/6 mice shortly after infection with the PMSS1 strain (CagA-positive). Most of these T cells were identified as $CD8\alpha\beta^+$ $TCR\alpha\beta^+$ T cells, which remained stable but decreased 3 months following infection, conversely $CD4^+$ $TCR\alpha\beta^+$ T cells expanded gradually after infection. CD8+ T cell count correlated inversely with bacterial burden. Increased numbers of CD3+ cells and CD8+ infiltration and total T cells were found in biopsies from *H. pylori*-infected patients than uninfected ones. CD8αß+ and $CD4^+$ expanded markedly in the infected group, and human gastric CD8αß+ T cells also showed T_{RM} phenotype. The progression of the infection was characterized by loss of the T_{RM} phenotype, a decrease in $CD8^+$, and an increase in $CD4^+$ T cells. *H pylori*-induced gastric $CD103^+$ $CD8^+$ T cells showed antigen-specific effector functions and gastric $CD8^+$ T_{RM} cells were specific to CagA[29].

Th22 cells, a subset of $CD4^+$, can produce IL-22, which reacts with IL-17 and tumor necrosis factor (TNF)[33]. In *H. pylori* infection, Th22 cells might have a pro-inflammatory effect, while Treg cells enable bacterial persistence by immunological tolerance. Yao et al[30] examined differences in Th22 and Treg cells, and levels of inflammation between *H. pylori*-infected and uninfected patients. They showed significantly higher serum levels of IL-22, transforming growth factor (TGF)-β, TNF-α, IL-4, IL-17A, and G17 (a marker of gastric inflammation) among *H. pylori*-infected patients than uninfected ones, while IFN-γ level was lower in the former. Results were comparable at the mRNA levels in the gastric mucosa. The percentages of the $IL\text{-}22^+$ $CD4^+$ and $Foxp3^+$ $CD4^+$ T cells in peripheral blood were also elevated in the *H. pylori* group. IL-22 and Foxp3 mRNA levels correlated positively with *H. pylori* colonization and gastric inflammation[30].

Sorini et al[31] examined the gastric microbial microenvironment and immune cell types in biopsies of patients who underwent gastric sleeve surgery and tested positive for *H. pylori* but lacked symptoms (i.e., asymptomatic infection) in comparison to uninfected individuals. They showed differences in the gastric microbiome composition between the groups, with amplified microbial function pathways toward metabolic processes and the immune system in the infected group. Epithelial cell signaling, antigen processing and presentation, Th17 cell differentiation, and IL-17 signaling increased in the infected group. *H. pylori* infection was related to the chronic activation of B cells, establishment of germinal centers, differentiation of plasmablasts, reduced $CD8^+$ T cells, and increased Th ($CD4^+$ T) cells[31].

Regulatory B cells (Bregs) are essential in modulating the immune responses mainly through the secretion of IL-10 and maintaining immune tolerance homeostasis and were linked to chronic infections[34], but their role in *H. pylori* infection remains elusive. A study of 112 adults who underwent upper digestive endoscopy (49.1% *H. pylori* positive by culture of gastric biopsies) analyzed peripheral blood specimens by flow cytometry and showed significantly reduced Bregs levels in *H. pylori*-infected patients than uninfected ones[32].

Additional studies assessed potential immunomodulation induced by *H. pylori* in gastric cancer patients and its association with survival and characterized the related immune profile[35,36], as well as the immune response that might implicate gastric *H. pylori i*nfection in the development of colorectal cancers[37].

While *H. pylori* is a well-established causative agent of gastric cancer, some reports showed that gastric cancer patients infected with *H. pylori*, particularly those in advanced stages, had better survival than uninfected ones, but the underlying immunological pathways remain unclear. A multiomics study examined the role of 73 *H. pylori*-related genes in tumor immunity in gastric adenocarcinoma, based on the Gene Expression Omnibus and The Cancer Genome Atlas database, and identified two different *H. pylori*-related gene mutation patterns with the markedly diverse tumor microenvironment (TME) infiltrating immune cell types[35]. Pattern C2 showed notable inflammation-promoting characteristics and strong immune activation. Activated B cells, CD8 T cells, eosinophils, T helper cells, mast cells, and macrophages were enriched in HPCluster C1, while activated CD4 T cells, neutrophils, and MHC-1, were amplified in HPCluster C2. Additional analyses revealed a group of gastric cancer patients with low-risk scores characterized by increased mutation burden, activation of immune responses and better 5-year survival as well as improved response to anti-PD-1/L1 immunotherapy[35].

Koizumi et al[36] studied survival rates among 491 advanced gastric cancer patients according to *H. pylori* infection and clinical-pathological parameters. They reported significant interactions between PDL-1 status and S-1 post-operative chemotherapy with *H. pylori* infection on relapse-free survival, suggesting better survival in the *H. pylori*-positive group. Among PD-L1 negative patients, but not PDL-positives, *H. pylori* was linked to higher survival rates. Among those who received S-1 chemotherapy coupled with surgery, the infected group had significantly improved survival. No significant interactions were found with other immune-related molecules[36].

Jin et al[38] showed that chimeric antigen receptor T cells (CAR T cells) expressing a neutrophil-activating protein (NAP), a potent proinflammatory antigen, from *H. pylori* activated endogenous bystander T-cell responses against solid cancers in mouse models. The administration of CAR(NAP) T cells in mice with various solid cancers controlled tumor growth and was related to higher survival rates compared to conventional mouse CAR T cells, irrespective of the target antigen, cancer type, and host haplotype, thus implying that CAR(NAP) T cells might have potential benefit[38].

Ralser et al[37] examined *H. pylori*-induced changes in the gut that might promote the development of colorectal cancer in mice (*Apc*-mutant mouse model for colorectal cancer and WT C57BL/6 mice) and in human samples. Gastric *H. pylori* infection promoted the development of intestinal and colonic tumors in *Apc*-mutant mice, induced a pro-inflammatory response in the intestine, and a decline in Treg cells. Additionally, *H. pylori* triggered signal transducer and activator of transcription 3 (STAT3), a pro-carcinogenic signaling pathway in the intestinal and colonic epithelium. Comparable immune and epithelial changes were evident in human colon specimens from *H. pylori*-infected patients[37].

VACCINES

Currently, a combination of antimicrobials and proton pump inhibitors is considered the best way to eradicate *H. pylori* infection and treat peptic ulcers and prevent gastric adenocarcinoma and gastric MALT lymphoma. However, the rapid emergence of antibiotic resistance of *H. pylori* is a concern, and alternative prophylactic and therapeutic vaccines are being evaluated. Multiple articles published in the last year describe different approaches in *H. pylori* vaccine development and evaluation of immunological and efficacy outcomes, which all were at pre-clinical development stages (Figure 1B)

H. PYLORI SUB-UNIT VACCINE CANDIDATES

Vaillant et al[39] showed that a subunit vaccine containing recombinant urease adjuvanted with cholera toxin induced a significant reduction in *H. pylori* gastric colonization of mice after challenge with *H. pylori,* compared to unvaccinated mice. The authors demonstrated that the protection against infection resulted from a sequence of events, including the production of GM-CSF by pathogenic Th17, further stimulation by GM-CSF of gastric epithelial cells to produce β defensin 3, which has the capacity to kill *H. pylori*. Inhibition of the biological activities of GM-CSF blunted the vaccine-induced reduction of *H. pylori* infection and vaccinated GM-CSF deficient mice only modestly reduced *H. pylori* infection, supporting the conclusion that GM-CSF-induced reduction of *H. pylori* infection burden is associated with an increased β defensin 3 gastric expression. These findings, in mice, suggest that adjuvanted recombinant urease (with cholera toxin) could be a candidate prophylactic and therapeutic *H. pylori* vaccine[39].

The outer membrane vesicles (OMVs) secreted by *H. pylori* represent a complex antigen proposed as another sub-unit candidate vaccine. Li et al[40] recently reported the construction of OMVs lacking small non-coding RNA (sncRNA), one of the OMV bacterial components. This approach followed the observation of the same scientists on the capability of *H. pylori* to reduce the host cell immune response to natural infection *via* sncRNAs delivered by OMVs. Indeed, intragastric delivery of deltasR-2509025 and deltasR-989262 OMVs in mice, significantly increased serum IgG and vaginal and stomach IgA levels compared to WT OMVs. Mice immunized with deltasR-989262 and deltasR-2509025 OMVs produced mixed Th1, Th2, and Th17 immune responses. Furthermore, DsR-2509025 and DsR-989262 OMVs were more effective at clearing gastric *H. pylori* colonization post-challenge than WT *H. pylori* OMVs[40].

FliD is a key colonization factor of *H. pylori*. Wei et al[41] assessed the immune responses to recombinant FliD (rFliD), showing increased levels of rFliD-specific IgG, IgM, and IgA among *H. pylori*-infected patients than healthy controls. The levels of rFliD-specific IFN-γ and IL-4 were higher among *H. pylori*-infected patients and displayed a Th1 dominant response, suggesting that rFliD might be a useful antigen candidate for *H. pylori* vaccine development[41].

ORAL LIVE-ATTENUATED VECTORS INCORPORATING *H. PYLORI* ANTIGENS

The oral live-attenuated vectors incorporating *H. pylori* antigens are designed to stimulate a specific mucosal immune response additionally to the systemic humoral and cellular response induced by other candidates.

Zhang et al[42] examined the immunogenicity and immunoprotective effect of an attenuated *Shigella* vector vaccine SH02 expressing the UreB-HspA fusion protein of *H. pylori* in a mouse model. Two delivery strategies were combined to effectively present the UreB-HspA fusion antigen to the mouse immune system. The first used a live bacterial vector, the live-attenuated *S. flexneri* 2a T32 Israti vaccine strain (renamed as SH02 when expressing the UreB-HspA fusion protein) developed and extensively used in 1960 to 1980s in Romania against shigellosis, and the second involved the administration of the booster dose either orally or subcutaneously after the primary oral doses. The control group received 3 oral doses of PBS at the same time intervals as the vaccine groups. Two weeks following the final vaccination, the mice were infected with live *H. pylori*, and eight weeks later, they were sacrificed, and samples were collected. Subcutaneous booster injection of the candidate antigen rUreB-HspA was superior to the oral booster in enhancing the level of the serum antigen-specific IgG antibodies and levels of IgG1/IgG2a/IgG2b subtypes. Specific fecal secretory IgA was detected in mice receiving the vaccine by both routes compared to controls without significant differences in the magnitude of response between the delivery routes. The subcutaneous boost also increased the proportion of CD4+CD154+ T cells that secrete IFN-g and IL-17A. Post-*H. pylori* challenge, the levels of *H. pylori* colonization were significantly reduced in both vaccine groups *vs.* the control group, indicating that the vaccine was preventive against *H. pylori* infection. Mice receiving the subcutaneous boost exhibited less gastric inflammation *vs.* the oral booster but without differences in *H. pylori* colonization[42].

Ghasemi et al[43] employed a new innovative Protective Immunity Enhanced *Salmonella* Vaccine (PIESV) vector strain (*S. Typhimurium* strain c12341) to express and orally deliver 4 conservative *H. pylori* antigens, the HpaA, Hp-NAP, UreA, and UreB. Immunization of mice with this vector and multiple antigens induced mixed Th1-, Th2-, and Th17-type immune responses. Strong and specific serum IgG and gastric mucosal IgA titers responses were elicited against each component of the cocktail vaccine. These findings indicated that the 4 *H. pylori* antigens synthesized and orally delivered by the PIESV vector strains induced both specific systemic and mucosal humoral immune responses. Importantly, this vaccine induced significant protection against *H. pylori* SS1 challenge with strong specific humoral and mucosal immune responses. Seven out of 10 immunized mice showed sterile protection and the other three mice showed a significant reduction in bacterial load versus the control mice[43].

Another orally delivered live-attenuated vector incorporating critical *H. pylori* antigens (Urease, CagA, VacA and NAP) is the M Cell-Targeting *L. lactis* Vaccine LL-plSAM-FvpE. Guo et al[44] reported the successful construction of this candidate vaccine based on a designed M cell-targeting surface display system for *L. lactis* to assist in delivering the vaccine antigen FVpE to the gastrointestinal tract. Mice vaccinated orally with LL-plSAM-FVpE could stimulate *H. pylori*-specific CD4+ T cells and antibody responses against urease, CagA, VacA, and NAP, and were protected against *H. pylori* infection. Significantly reduced *H. pylori* burden and urease activity were found following LL-plSAM-FVpE compared with LLplSAM or SAM plus PA. In 8 out of 10 vaccinated mice no *H. pylori* colonization was found in the stomach. Mucosal secretory IgA antibodies against *H. pylori* were detected in the gastrointestinal tract post-oral vaccination with LL-plSAMFVpE suggesting that the protection of LL-plSAM-FVpE against *H. pylori* may be associated with antibody-mediated humoral immunity against multiple virulence factors of *H. pylori*[44].

IN SILICO VACCINE DESIGN

Al-Eraky et al[45] used the reverse vaccinology approach to identify antigens that can serve as vaccine candidates against *H. pylori* and evaluated their prophylactic effect in BALB/c mice. Four antigenic peptides were prioritized as potential vaccine candidates (CagA1 and CagA2 antigenic epitopes, a putative outer membrane protein (SabA), and vacuolating cytotoxin (VacA). The peptides were subcutaneously administered to mice emulsified with Freund's adjuvant. The immunized mice were challenged orally with *H. pylori*. IgG, IgA, IL-4, and IL-17 were detected in mice sera. The SabA protein was the most immunogenic antigen inducing significantly higher levels of specific IgG, IL-4, and IL-4/IL-17 ratio in vaccinated mice compared with the control groups that received either PBS or adjuvant. Histopathological examination of gastric tissue showed a protective effect in the vaccine groups (each peptide) compared to adjuvant and PBS groups. Further research with additional immunological and efficacy parameters is needed to confirm the value of SabA protein as a subunit vaccine candidate alone or coupled with additional antigens[45].

Chehelgerdi et al[46] studied the CagW protein as a potential *H. pylori* subunit vaccine candidate using immunoinformatic and computation methods. They amplified and cloned the *cag*W gene (part of the pathogenicity island) into pcDNA3.1 (+), documented stability and in vitro expression, and injected it into the muscles of BALB/c mice. pcDNA3.1 (+)-*cag*W-immunized mice showed an increase in both the number of lymphocytes that dispersed throughout the body and the quantity of IFN-γ, IL-2, IL-4, and IL-12 that was produced than control-plasmid-immunized mice. Moreover, pcDNA3.1 (+)-*cag*W vaccine effectively protected mice against *H. pylori* infection after intraperitoneal challenge with live *H. pylori*. Through a comprehensive immunoinformatic approach and modeling, the investigators identified epitopes and conditions with the best capabilities to enhance the protective efficacy of this *cag*W-based candidate vaccine[46].

Conflict of Interest

The authors declare no conflict of interest.

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