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INFLAMMATION, IMMUNITY, AND VACCINES

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Abstract – *Helicobacter pylori,* the most common gastric infection in the world, causes gastritis, peptic ulcers, and gastric cancer. Innate and adaptive immune responses are induced after *H. pylori* infection. However, it achieves long-term colonization through immune evasion. Understanding the mechanisms of inflammation and immunity induced by *H. pylori* is crucial for developing preventive and therapeutic vaccines. This review captures the major advancements in *H. pylori*-induced inflammation, immunity, and vaccine development from April 2023 to March 2024. Several studies have revealed new regulators (CDK1 and NOD1) of *H. pylori*-induced inflammation through NF-kB and non-NF-kB pathways and participation of *H. pylori* CagA and HtrA. More antimicrobial agents are found to have protective functions by ROS, neutrophil, and NK cell activation. TINAGL1 is reported to negatively modulate DCs and Th1 cells by *H. pylori* infection in an IL-1β dependent way. Moreover, CD4+IL-17A+FOXP3+ T cells show a proinflammatory function modulated by DCs and IL-6. *H. pylori* vaccine studies focus on the in-silico design, construction, and in vitro and in vivo evaluation of new vaccine candidates with different strategies. These studies demonstrate promising immunogenicity and efficacy results, underscoring the potential for future vaccine development.

Keywords: *Helicobacter pylori*, Inflammation, Immunity, Vaccine.

Abbreviation: AlpB: Hop family adhesin; CagA: Cytotoxin-associated gene A; CDK1: Cyclin-dependent kinase 1; DC: Dendritic cells; FlaA: Flagellin A; gGT: γ-glutamyl-transferase; GSH: Glutathione; HpaA: *H. pylori* adhesin A; HPCM: H. pylori-infected gastric cells; HtrA: High-temperature requirement A; IAV: Influenza A virus; MALT: Mucosa-associated lymphoid tissue; MECU: Multi-epitope chimeric antigen; MND: Metal-based nanodrugs; moDCs: monocyte-derived dendritic cells; MS: Mesoporous shell; NapA: Neutrophil-activating protein A; NK: Natural killer; NKG2D: Natural killer group 2, member D; NOD1: Nucleotide-binding oligomerization domain 1; PRR: Pattern recognition receptors; ROS: Reactive Oxygen Species; SabA: Sialic acid-binding adhesin; SLN-A: Solid lipid particles containing monophosphoryl lipid A; SNP: Single-nucleotide polymorphism; TINAGL1: Tubulointerstitial nephritis antigen-like 1; TLR: Toll-like receptor; UreB: *H. pylori* Urease B subunit.

INTRODUCTION

Helicobacter pylori, the most prevalent gastrointestinal pathogen, infects humans at an early age and becomes persistent for decades. This chronic infection causes various gastric diseases, including chronic gastritis, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma[1]. *H. pylori* infection induces a strong innate and adaptive immune response in the gastric mucosa, fighting against bacterial colonization. After being recognized by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), *H. pylori* triggers the infiltration of innate phagocytotic immune cells and the secretion of inflammatory cytokines, which further activates an adaptive $CD4^+$ T helper (Th) 1 and Th17, $CD8^+$ T cell response, and specific antibody production[2].

However, the immune evasion strategies developed by *H. pylori* enable the long-term persistence of the bacterium, which results in the progress of gastric lesions and even gastric cancer[2]. Several virulence factors of *H. pylori*, such as the cytotoxin-associated gene A (CagA), γ-glutamyl-transferase (gGT), neutrophil-activating protein A (NapA), and urease, participate in its immune invasion and pathogenesis by modulating the immune response during *H. pylori* infection[3]. Understanding the mechanism of inflammation and immune response against *H. pylori* is essential for developing an efficacious vaccine and the prevention of *H. pylori*-associated diseases.

METHODS

After searching for publications from April 2023 till March 2024 that are related to *H. pylori* in the medical bibliographic database, we selected the publications by keywords (inflammation, immune response, vaccine, pro-inflammatory cytokine, virulence factors, and so on) and distributed them in the three parts including immunology, inflammation, and vaccine. We removed unqualified and unrelated studies by abstract assessment, by comparing several similar studies, and by journal reputation. This review summarizes the recent most relevant publications on *H. pylori*-related inflammation, immunology, and vaccines from April 2023 till March 2024.

INFLAMMATION

Inflammation-Related Signaling Pathways

Inflammation promoted by NF-kB activation during *H. pylori* plays a vital role in gastric tumorigenesis. Zhu et al[4] reported the increased Cyclin-dependent kinase 1 (CDK1) expression in mouse gastric tissue after *H. pylori* infection and proved that CDK1 linked NF-kB and β-catenin activation. Furthermore, by using a conditional CDK1 knockout mouse model ($Krt19^{Cre}/Cdk1^{flox/flox}$) they observed less inflammation and reduced β-catenin expression in murine organoids in the CDK1-deleted group compared to the no-deleted group after *H. pylori* infection. Sharafutdinov et al[5] focused on the *H. pylori* high-temperature requirement A (HtrA) gene's contribution to gastric disease. By analyzing the *HtrA* gene single-nucleotide polymorphisms (SNPs) and correlating them with gastric disease outcome, they found a serine/leucine exchange in the protease domain at amino acid position 171 (171S/L) that was significantly associated with gastric disease. They further showed an enhanced E-cadherin cleavage and CagA translocation, an increased NF-kB and β-catenin activation, and higher IL-8 secretion after 171L-type HtrA strains infection experiments *in vitro*. These findings revealed new mechanisms of NF-kB-mediated gastric inflammation caused by *H. pylori* infection.

Except for the NF-kB signaling pathway, there are other pathways that participate in the inflammation process during *H. pylori* infection. Recently, Tran et al[6] showed that the level of *IL-18* expression was significantly higher in mice gastric epithelial cells compared to immune cells after infection. They postulated that IL-18 responses were related to nucleotide-binding oligomerization domain 1 (NOD1), which responds to muropeptides delivered by the T4SS[7]. Therefore, they infected the *NOD1* knockout AGS cell line and *IL-18* deficient mice and showed a loss of IL-18 response in the absence of NOD1. Moreover, IL-18 production was reduced in AGS cells infected with T4SS-deficient strains compared to the WT strain, suggesting that IL-18 response during *H. pylori* infection depends on NOD1-T4SS[6]. By comparing IL-18 expression *in vitro* with/without NF-kB and caspase-1 inhibition, they demonstrated that NOD1 activates caspase-1, not NF-kB, to promote IL-18 induction, which is essential in *H. pylori*-induced inflammation. This study is an important continuation of earlier work from the group in *H. pylori*-induced NOD1 induction and contributes nicely to a better understanding of the complex immune response towards *H. pylori.*

TLRs

It was recently reported that TLR6 expression in gerbils gastric epithelial cells was initially upregulated but then downregulated after long-term *H. pylori* infection, together with decreased levels of the pro-inflammatory cytokines IL-1β and IL-8[8]. After being administered with a TLR6 agonist after chronic *H. pylori* infection, gerbils' gastric epithelial cells were observed to in-

crease IL-8, Ly6G, and CD11b expressions with less *H. pylori* colonization, indicating that the activation of host immune responses through TLR6 pathways supported the clearance of *H. pylori* infection[8]. They conclude that suppressed expression of TLR6 in gastritis patients' gastric mucosa with *H. pylori* infection may make TLR6 a promising target for immunotherapy to *H. pylori* infection, although it remains to be shown to which extent a receptor that is downregulated can be targeted.

Reactive Oxygen Species (ROS)

H. pylori induces the production of ROS through the activation of ROS-related immune response, which stresses the host gastric epithelial cells with oxidative damage and plays an important role in *H. pylori*-related carcinogenesis[9]. The antioxidant glutathione (GSH) level in gastric tissue, which is essential for the detoxification of ROS, is reduced by *H. pylori* infection[9]. Baskerville et al[10] quantified the total GSH and oxidized disulfide dimer GSSG levels in *H. pylori*-infected AGS cells. They observed a depletion of total GSH and similar levels of GSSG after infection, showing the oxidation-independent host GSH depletion by *H. pylori*. Moreover, they detected much higher GSH and lower Cys-Gly levels in cells infected by gGT mutant *H. pylori* strain than by the wild-type strain, demonstrating the contribution of gGT of host GSH depletion through Cys-Gly dependent manner during *H. pylori* infection. Thereby, the study adds another piece of data to confirm the importance of this virulence factor to *H. pylori* metabolism and interaction with the most.

However, ROS are released by neutrophils initially to kill pathogens such as *H. pylori*[2]. Yu et al[11] reported that an acid-responsive ROS nanogenerator could induce ROS triggered by ultrasound and significantly reduce *H. pylori* colonization and gastric inflammation in mice through a ROS-dependent manner with no evident toxicity. Furthermore, the microbiota of mice under treatment for *H. pylori* eradication exhibited a similar abundance of bacteria in the intestine to that of naive control, while it was significantly reduced by triple antibiotic treatment. The results suggested the potential of an acid-responsive ROS nanogenerator as a treatment without disrupting the homeostasis of the intestinal flora. At the same time, increased intracellular ROS was observed by a study focused on the effect of linolenic acid-metronidazole (L1a-Met) inhibiting the colonization of *H. pylori*[12]. After L1a-Met treatment, *H. pylori* cells upregulated the expression of anti-oxidation-related genes, SodB and MdaB, which suggested the oxidative stress-related killing toward *H. pylori* by L1a-Met and provided the future of L1a-Met as a broad-spectrum anti-*H. pylori* drug. The recent findings of ROS-correlated inflammation and ROS-dependent killing reflect the dual character of ROS in *H. pylori* infection. The antibacterial effect of L1a-Met in metronidazole-resistant strains with high prevalence observed globally remains to be shown. L1a-Met might become an important alternative treatment option.

Inhibition of CagA

Besides broad-spectrum antibacterial agents, several studies focused on small-molecule inhibitors targeting CagA translocation. Recently, Lettl et al[13] identified a high-potency Cag T4SS inhibitor and observed the growth inhibition of *H. pylori* by culturing the bacteria with mitochondrial complex I or III inhibitors. They further revealed the target proteins of respiration inhibitors, NuoB and NuoD, by finding the decreased sensitivity against respiration inhibitors with *H. pylori*-NuoB and *H. pylori*-NuoD gene mutant infection. Furthermore, after molecular modeling of the *H. pylori* complex I quinone binding cavity, they tested the compound sensitivity in correspondent *H. pylori* variants and demonstrated the altered sensitivity of *H. pylori* toward complex I inhibitors. These observations indicated the therapeutic potential of complex I inhibitors as an antibacterial agent against *H. pylori* by its unique quinone-binding pocket. SHP1 has been found to dephosphorylate CagA and attenuate the pathogenesis of *H. pylori*[14]. Chen et al[15] applied the agonist of SHP1, Sorafenib, in *H. pylori*-infected mouse model. They showed reduced neutrophil infiltration, relieved mucosal damage in mouse gastric samples, and decreased serum IL-8 secretion. The data above provided more possibilities about the potential of developing CagA translocation inhibitors for *H. pylori* treatment. While the protective effects of Sorafenib are compelling, it is still unclear in which clinical setting or indication such an approach may be used.

Probiotics

Apart from antimicrobial agents, the applications of probiotics also assist *H. pylori* eradication. Probiotics, *Lactobacillus acidophilus* NCFM and *Lactiplantibacillus plantarum* Lp-115, were found to inhibit the adhesion of *H. pylori in vitro* and downregulate the IL-8 and TNF-α expression in AGS cells[16]. They also exhibited an *H. pylori* colonization suppressive effect *in vivo* and a reduced IFN-γ expression in mice stomachs, suggesting the inhibition of host inflammatory response by probiotics. Additionally, a spore-forming probiotic, *Weizmannia coagulans* (strain BCF-01), was found to suppress inflammatory cytokine expressions, such as IL-1β and TGF-β *in vivo* and *in vitro* after *H. pylori* infection and a higher abundance of *Bacillus*, *Lactobacillus*, and some potentially beneficial genera (such as *Akkermansia*) by microbiota analysis[17]. Moreover, another study[18] that infected with the gastric commensal *Cutibacterium acnes* (*C. acnes*) first and *H. pylori* next in mouse model exhibited reduced colonization, lower *Ifn-γ*, *Tnf-α*, and *Il-17a* expression. The above results showed new evidence that specific microbiomes affect inflammatory and cancer associated cytokines in *H. pylori* pathogenesis.

Gut-Brain Axis

Kandpal et al[19] treated the neuroblastoma and neuron-astrocyte co-cultured cells with conditioned media from *H. pylori*-infected gastric cells (HPCM). They found significantly elevated expression of pro-inflammatory cytokines, chemokines, STATs regulatory molecules, and ROS reaction in neuroblastoma cells. After inhibiting STAT3 in HPCM, they observed a reduced TNF-α protein expression in the gastric cell line, suggesting the control of gastric inflammatory response. These results indicated that *H. pylori* may induce gastro- and neuro-inflammation through STAT3 activation, which gave further insights into *H. pylori*-related gut-brain axis.

INNATE AND ADAPTIVE IMMUNE RESPONSE

Innate Immune Cells

Innate immune cells such as dendritic cells (DC), neutrophils, and natural killer (NK) cells are recruited and play different functions during *H. pylori* infection. Teng et al[20] reported that tubulointerstitial nephritis antigen-like 1 (*TINAGL1*) expression increased in the gastric mucosa of *H. pylori*-positive patients and mice. *In vitro* experiments in mouse and human cell lines showed increased *TINAGL1/Tinagl1* expression after *H. pylori* infection in the presence of IL-1β and a positive correlation between *TINAGL1/Tinagl1* and *IL1B/Il1b* expression. They further exhibited diminished induction of *TINAGL1/Tinagl1* expression and TINAGL1 production by *H. pylori* and IL-1β in Il1r1$^{-/-}$ mice, which suggested that *H. pylori* and IL-1β synergistically induce TINAGL1. Reduced *H. pylori* colonization, increased monocyte-derived DCs (moDCs) number, and IFN-γ production were shown in Tinagl1$^{-/-}$ mice compared to WT mice. Conversely, the colonization significantly increased, moDCs number and IFN-γ production decreased after the injection of TINAGL1 into these mice. After screening chemokine gene expression in Tinagl1$^{-/-}$ mice, they found that the expression of *Ccl21* was higher than in WT mice. Furthermore, increased CCL21 production in the gastric mucosa of Tinagl1$^{-/-}$ mice and decreased CCL21 production in the mutant mice injected with TINAGL1 were shown. They also demonstrated higher moDC migration after co-culturing the supernatant from *H. pylori*-infected primary GECs of Tinagl1$^{-/-}$ mice than that of WT mice, which was abolished by neutralizing CCL21 and CCR7 antibody treatment. These results suggested that the TINAGL1-CCL21-CCR7 axis contributes to moDC accumulation within the gastric mucosa during *H. pylori* infection. By transferring CD4$^+$ T cells from *H. pylori*-infected WT donors into Tinagl1$^{-/-}$ recipients, they effectively reduced *H. pylori* colonization compared to that in WT recipients receiving the same CD4$^+$T cells, indicating that TINAGL1-mediated inhibition of *H. pylori*-specific CD4$^+$ T cells leads to increased bacterial colonization. However, transferring CD4$^+$ T cells from *H. pylori*-infected Ifnγ $^{-/-}$ donors into Tinagl1$^{-/-}$ recipients abolished the reduction observed above, suggesting that TINAGL1 promotes gastric *H. pylori* colonization by inhibiting IFN-γ production by *H. pylori*-specific Th1 cells.

The receptor of natural killer group 2, member D (NKG2D) system, is widely expressed on NK cells, cytotoxic T lymphocytes, and γδ T cells, which is essential for mucosal homeostasis and could be modulated by *H. pylori* infection[21]. Anthofer et al[22] reported a lower expression of the NKG2D-encoding gene, MICB, in human gastric tissue of *H. pylori*-positive gastritis and stomach adenocarcinoma patients compared to healthy controls. They also found lower NKG2D ligand expression in gastric epithelial cells after *in vitro* infection with *H. pylori* isogenic mutants, ΔcagA, ΔcagL, and ΔvacA, which suggested that proteolytic shedding of NKG2D ligands depends on *H. pylori* virulence factors and added more evidence to the participation of NKG2D-related immune cells to *H. pylori*-induced inflammation and carcinogenesis.

T Cells

Guo et al[23] analyzed stomach T cells from *H. pylori*-positive (Hp^+) patients with gastritis and demonstrated a significantly higher number of $CD4^+IL\text{-}17A^+FOXP3^+$ T cells in human *H. pylori*-positive gastric mucosa tissue. Furthermore, they compared functional characteristics of $IL\text{-}17A^+$ and $IL\text{-}17A^-$ populations in $CD4^+FOXP3^+$ T cells in human gastric tissue. They showed higher TNF-α, IFN-γ, and IL-23 expression in the $IL\text{-}17A^+$ population than in the $IL\text{-}17A^-$ population. These results suggested that $CD4^+IL\text{-}17A^+FOXP3^+$ T cells exhibit a strong proinflammatory capacity. Given that DCs, macrophages, and IL-6 promote T cell activation, they assessed IL-6 expression levels in various human gastric cell subpopulations and observed increased IL-6 expression levels in $CD11c^+$ DCs and $CD68^+$ macrophages in the blood of Hp^+ patients. After sorting DCs and macrophages from Hp^+ patients and co-culturing with various strains of *H. pylori*, they showed lower IL-6 protein and mRNA levels with a ΔCagA *H. pylori* strain compared to the WT strain. This IL-6 activation of DCs and macrophages by WT *H. pylori* co-culturing was suppressed by blockage of the NF-κB pathway, which indicated that $CagA^+$ *H. pylori* upregulate IL-6 expression in $CD11c^+$ DCs and $CD68^+$ macrophages through the NF-κB pathway. After co-culturing $FOXP3^+$ T cells from PBMC with $CagA^{+/-}$ *H. pylori*-infected DC or WT *H. pylori*-infected DC with and without anti-IL-6 treatment, they found that IL-17A production from the ΔCagA group and from the IL-6-blocked group was significantly decreased. The data suggested that DCs/macrophages induce IL-17A expression in $CD4^+FOXP3^+$ T cells in CagA and IL-6 dependent way. Moreover, they revealed a positive correlation between $CD4^+IL\text{-}17A^+FOXP3^+$ T cells' number and *TNF*, *IFN-γ*, and *IL-8* gene expression in the Hp^+ patient's stomach. Also, they showed that the number of gastric $CD4^+IL\text{-}17A^+FOXP3^+$ T cells had a step-by-step increased pattern from patients with non-atrophic gastritis to atrophic gastritis and atrophic gastritis to intestinal metaplasia. Therefore, these findings offer essential insights into the differentiation of inflammatory $IL\text{-}17A^+FOXP3^+CD4^+$ T cell population through the inflammation and immune response caused by *H. pylori* infections, thereby guiding the effector immune response and aggravating gastritis. Interestingly, a similar mechanism of $CD4^+$ Treg transdifferentiation towards pathogenic $Th17^+$ cells has also recently been described in the intestine and colon upon *H. pylori* infection[24].

Another study[25] focused on *H. pylori* treatment with metal-based nanodrugs (MNDs), which have antibacterial functions by direct destruction of bacterial cytoskeleton, production of bacteriostatic metal ions, and generation of ROS. They reported that *H. pylori* colonization was significantly downregulated in mice treated with MND with less gastric inflammation. The number of gastric IFN-γ and IL-17 secreting cells was lower, and the number of proinflammatory cytokines IL-6, IL-17, and IFN-γ secretion was lower in the MNDs treated group compared to the control group, which was similar to classic *H. pylori* eradication treatment group. In addition, they demonstrated an increased proportion of $CD4^+$ T cells in mice stomachs 3 days after MNDs treatment, which went back to the same level as the naïve group 7 days after MNDs treatment. They did not find significant change in weight, inflammatory lesions from other organs, and intestine microbiome after MNDs treatment in mice, which suggested that MNDs have great potential to remove *H. pylori* infection through $CD4^+$ T cell response with low toxicity and microbiota disturbance.

He et al[26] reported more $CD8^+$ T cells than $CD4^+$ T cells in *H. pylori*-infected human gastric tissues. At the same time, they summarized 3 most frequent HLA-I genotypes from the Chinese Han population in the allele database and predicted and synthesized 10 peptides of *H. pylori* Urease B subunit (UreB) protein based on each HLA-I type above. Then, they stimulated PBMC from *H. pylo-*

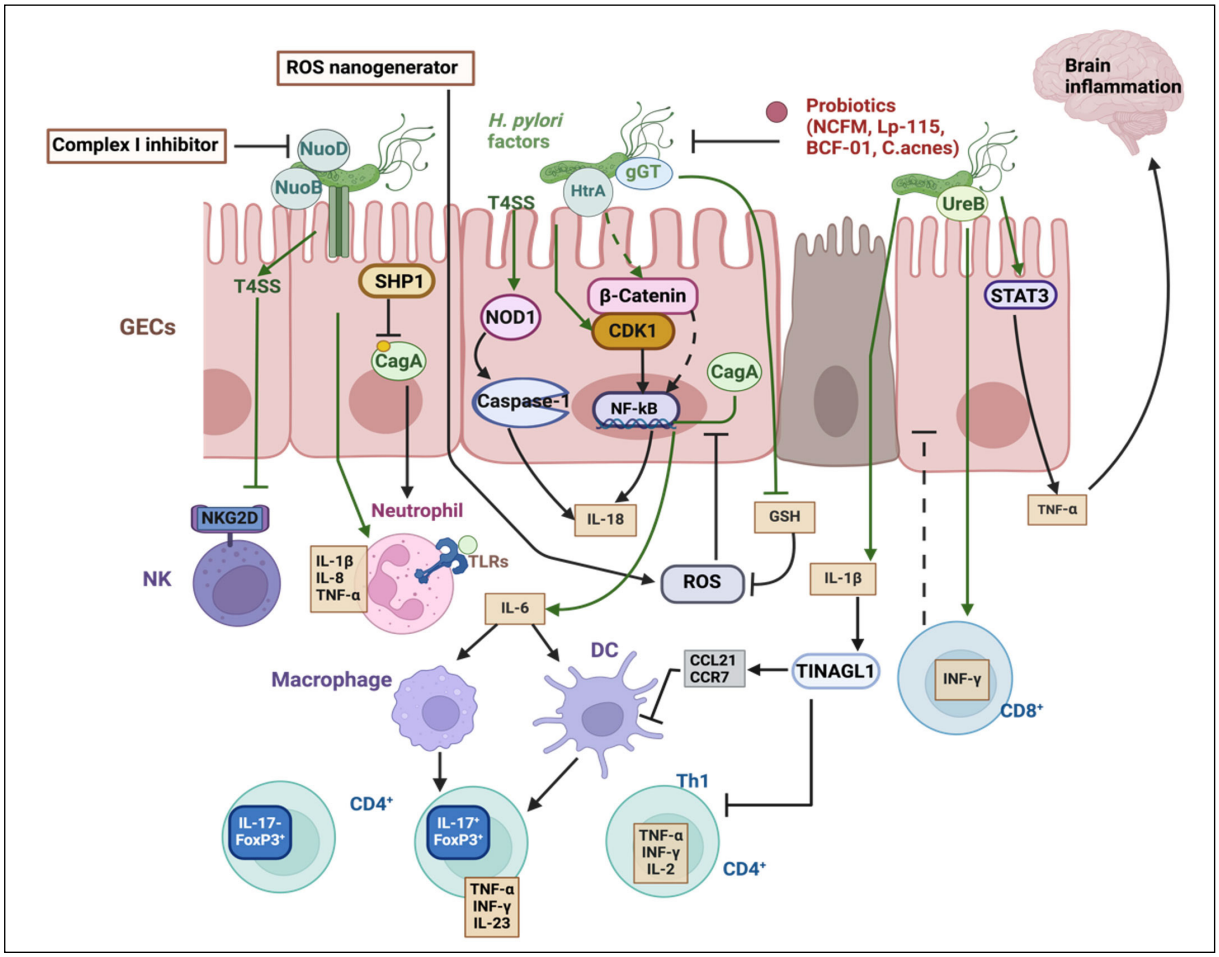

Figure 1. Recent findings of immune response to *H. pylori.*

ri-infected individuals with 3 peptide pools *in vitro* and found significantly higher IFN-γ secretion by $CD8^+$ T cells after peptide pool re-stimulation compared to non-stimulation in peptide responding individuals. Furthermore, they picked the most potent responder peptide from each peptide pool and tested the re-stimulation result in 506 *H. pylori*-positive individuals whose clinical information was collected and divided into groups with and without gastric symptoms. The UreB C-1 (UreB5-13) specific $CD8^+$ T-cell responses were more likely to occur in *H. pylori*-infected individuals without gastric symptoms and may reduce the risk of appearance in gastric symptoms, which could thus be a future candidate for vaccine study. The current findings of *H. pylori*-related inflammation and immune response are summarized in Figure 1.

VACCINES

H. Pylori Urease-Related Vaccine Studies

Skakic et al[27] formed nanocapsules derived by the recombinant UreA, mesoporous shell (MS), and solide core/mesoporous shell (SC/MS), which were used for immunization. Serum IgG1 increased significantly in the UreA-nanocapsule groups compared to the control group post-challenge. Regarding *H. pylori* burden, mice vaccinated with UreA-SC/MS and TiterMax adjuvant had significantly lower colonies compared to controls. The number of $CD4^+$ T cells was higher in most vaccinated groups. However, only UreA-SC/MS and UreA-MS combination groups (with/without TiterMax) had upregulated $CD8^+$ T cell response. Meanwhile, scholars[28] also synthesized novel solid lipid particles containing the adjuvant lipid monophosphoryl lipid A (SLN-A) and produced a vaccine by binding SLN-A with a DNA encoding UreA of *H. pylori* (Lipoplex). Serum IgG2C levels

in Lipoplex prime-UreA protein boost and Lipoplex prime-Lipoplex boost groups were significantly increased, together with an upregulated number of $CD4^+$ T cells. However, *H. pylori* burdens did not change in any experimental group compared to the control. Katsande et al[29] engineered spores of *Bacillus subtilis* (*B. subtilis*) to display the *H. pylori* full-length *ureA* gene and a segment of *ureB* termed $ureB^{CT}$ on the spore surface. Whole spore ELISA was used to demonstrate the display of antigens on the spore coat of *B. subtilis* (CotB). CotB-UreA and CotB-UreBCT groups exhibited significantly higher serum IgG and sIgA and lower bacterial colonies. The data suggested the protection induced by nanoparticle and spore vaccines against *H. pylori*, which induced UreA or UreB-specific immune response.

The *UreH* gene was inserted into the pCI-neo expression vector and formed with an alginate nanoparticle (alginate/pCI-neo-*UreH*) by Kaveh-Samani et al[30]. Alginate/pCI-neo-*UreH* oral immunization significantly reduced bacterial burdens in stomach tissues compared to the control group with higher serum antigen-specific IgG antibody titers and higher proinflammatory cytokine secreted by splenocytes.

Other *H. Pylori* Protein-Related Vaccine Studies

Ji et al[31] developed a vaccine candidate based on a multi-epitope chimeric antigen (MECU) containing a structural scaffold from UreB and B cell epitopes from the movement-related protein flagellin A (FlaA), *H. pylori* adhesin A (HpaA), hop family adhesin (AlpB), and sialic acid-binding adhesin (SabA). The serum IgG levels were significantly higher after oral immunization with MECU and CTB adjuvant. Vaccinated mice, after the challenge, exhibited significantly increased serum IgG and sIgA levels, IFN-γ and IL-17 production in the splenocytes, and decreased *H. pylori* colonization.

Nie et al[32] recently employed influenza A virus (IAV) vectors to express NapA, including influenza virus A/WSN/33 (WSN)-NapA and A/Puerto Rico/8/34 (PR8)-NapA. Significantly lower virus titers were observed in the WSN-NapA group compared to the control group. Fatal viral infection was shown in the PR8-NapA group, suggesting that WSN-NapA was safer for the primary immunization vaccine. The mice exhibited NapA-specific humoral immune response (serum NapA IgG and gastric sIgA) and significantly higher IFN-γ and IL-17 secretion in $CD4^+$ T cells in the spleen after immunization. Mice showed significantly lower colonies and less inflammation than the controls in prophylactic and therapeutic-challenged settings.

Zhang et al[33,34] focused on the *Lactococcus lactic acid* (*L. lactis*) vaccine, which employed *L. lactis* as a novel oral vaccination delivery vehicle. WAE gene (Urease, HpaA, HSP60, and NAP) was inserted into a synthetic plasmid (plSAM-WAE) and transformed into *L. lactis* to construct LL-plSAM-WAE that specifically targets microfold cells[33]. *In vivo* evaluation showed antigen-specific IgG response and significantly higher inflammatory cytokine secretion from spleen $CD4^+$ T cells in the LL-plSAM-WAE group compared to the controls. Less bacterial load, stronger humoral (serum IgG and sIgA), and cellular (IFN-γ, IL-4, and IL-17) immune response in the LL-plSAM-WAE group suggested the protection after *H. pylori* infection. The same group[34] also generated LL-plSAM-FAdE (Urease, CagL, HpaA, and Lpp20) and showed similar protection in the immunized BALB/C mouse model.

These results indicated that different *H. pylori* protein, single or multiple, are promising immunogenic candidates for the *H. pylori* vaccine with different delivery options (Table 1).

However, while it is certainly encouraging that such diverse approaches can lead to protection, this also raises the question of why none of these approaches seem to be further advanced towards a preclinical candidate of even clinical trials. The fact that many different antigens and vaccination approaches have shown reduced colonization in mice, but only a single approach has ever shown at least some protection in humans[35], should raise doubts about the suitability of mouse models for developing and prioritizing vaccine candidates against *H. pylori*, and the quality of the immune correlates being analyzed. The field clearly lacks predictive correlates of protection, as well as more advanced animal models with better predictivity towards human trials.

CONCLUSIONS

In conclusion, *H. pylori* remains a significant global health challenge due to its ability to cause chronic gastritis and gastric cancer, employing sophisticated immune evasion mechanisms to ensure persistence. Recent research has provided valuable insights into the immune responses in-

TABLE 1. RECENT *H. PYLORI* VACCINE DEVELOPMENT.

Antigen	Type	Adjuvant	Mouse model	Method	Times	Interval	Infection strain	Ref
UreA	Nanocapsule vaccine	TiterMax	C57BL/6	NA[1]	2	2 weeks	SS1	26
UreA	Solid lipid nanoparticles DNA vaccine	–	C57BL/6	Subcutaneous administration	2	2 weeks	SS1	27
UreA or UreB	Spore coat vaccine	–	C57BL/6	Oral administration	4	2 weeks	HP34	28
UreH	Alginate nanoparticle DNA vaccine	–	BALB/c	Oral administration	3	15 days	SS1	29
CTB and UreI	DNA vaccine	–	BALB/c	Intramuscular administration	3	2 weeks	SS1	30
NapA	Influenza A virus (IAV) vectors vaccine	–	BALB/c	Intranasal administration	2	3 weeks	SS1	31
Urease, HpaA, HSP60, and NAP	*L. lactis* multi-epitopes vaccine	–	BALB/c	Oral administration	4	1 week	SS1	32
Urease, CagL, HpaA, and Lpp20	*L. lactis* multi-epitopes vaccine	–	BALB/c	oral administration	4	1 week	SS1	33

NA: not available.

duced by *H. pylori*. New regulators of *H. pylori*-induced inflammation *via* NF-kB and non-NF-kB pathways have been identified, along with the involvement of *H. pylori* virulence factors, potentially facilitating the development of future therapeutic targets. Advances in understanding the protective functions of antimicrobial agents have expanded our knowledge of host-pathogen interactions. Furthermore, new findings on inflammatory and regulatory effects highlight the importance of immune cells in *H. pylori* infection. Promising progress in vaccine development, particularly through *in-silico* design and comprehensive evaluations of new candidates, demonstrates potential effective prevention approaches. Collectively, these findings pave the way for innovative strategies to combat *H. pylori* infections and mitigate their associated health burdens.

Conflict of Interest

The authors have no conflicts of interest to declare. Both co-authors have seen and agreed with the manuscript's contents, and there is no financial interest to report. We certify that the submission is original work and is not under review at any other journal.

Authors' Contribution

Ruolan Gong is responsible for searching for literature, summarizing, and writing the manuscript. Markus Gerhard is responsible for reviewing and editing the manuscript.

Ethics and Informed Consent

Not applicable due to the type of study.

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AI Disclosure

AI-associated technology is not used in the preparation of this work.

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