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Review - Human and Animal Health

Immune Response and Therapeutic Vaccination against Helicobacter pylori

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HIGHLIGHTS

- The interaction between innate and adaptive immune responses against *H. pylori.*
- The virulence factors of *H. pylori* to evaluate the susceptibility of the infection.
- New treatment and vaccination approaches for *H. pylori* relating possible mechanisms.
- Mechanisms shaping innate and adaptive immune responses considering genetics.

Abstract: *Helicobacter pylori (H. pylori)* is a gram-negative bacterium, considered one of the significant discoveries about 40 years ago, and was isolated and cultured from the human stomach. *H. pylori has* infected more than half of the human population, making it one of the most well-known human pathogens. The front line of immune response starts with innate recognition of *H. pylori* and its mediators and intracellular signaling by gastric epithelial cells in which they recognize and respond to bacterial products such as flagella, lipopolysaccharides, and peptidoglycan. The inflammatory response is followed by the recruitment of various cells of the innate and adaptive immune system. Cytokines including IL-12, IL-23, and TGF-β direct the polarization of CD4+ T helper cells to Th1, Th17, and Treg, respectively. The clinical symptoms that may occur as a result of *H. pylori* infection linked to the virulence factors of the bacteria, the genetic factors of the host, and the immune responses. Specific antigens have been found as part of these crucial virulence factors. The specific antigens may play a role in the development of an effective vaccine to eradicate *H. pylori* infection. Innate and adaptive immunity and genetic factors have an important place in understanding the host response mechanisms, elucidating the pathogenesis of the disease, and developing new targeted

therapy approaches. Thus, the aim of this study is to understand immune responses and investigate the potential therapeutic vaccination against *H. pylori.*

Keywords: Adaptive immunity, *Helicobacter pylori*, immune response, innate immunity, vaccine, virulence factors.

INTRODUCTION

H. pylori, a spiral-shaped bacterium strain, was successfully isolated and cultured from a human stomach by Barry Marshall and Robin Warren in 1982 [1]. Experiments with volunteers followed by self-ingestion of bacterial suspension experiments by Marshall and Morris showed that these bacteria were able to colonize the human stomach, thereby inducing inflammation of the gastric mucosa [2]. *H. pylori* was originally named Campylobacter pyloridis. After sequencing the gene in 1989, it was revealed that it does not belong to the Campylobacter class. For this reason, it has been classified into a new class as *H. pylori.* The Greek word "Helico" means "spiral" or "coil" [3]. *H. pylori* is a spiral-shaped, coiled gram-negative bacterium. It forms 1 mm diameter non-pigmented colonies on chocolate agar and blood agar in three days at 37 °C in microaerophilic environment. *H. pylori* is very mobile and produces copious amounts of urease. Due to these features, they can survive in the acidic and mucous environment of the stomach [4]. *H. pylori* enters the human body through the mouth and moves through the digestive tract. It activates the immune system by infecting the first line of the stomach or small intestine. This review focuses on the interaction of *H. pylori* with the innate immune system, adaptive immune response mechanisms, and a range of virulence factors with genotype. In addition, understanding the immune response mechanisms is important both for elucidating the pathogenesis of the disease and for the development of new treatment approaches.

Innate immunity in *H. pylori*

The discovery of *H. pylori* by Barry Marshall and Robin Warren in 1982 marked the beginning of a new era. Despite human immune responses to this pathogen, it can survive for a long time in the gastric mucosa. Since the inflammatory response (inflammation) decreases in its favor, it forms the basis of the persistence of the infection in humans. Microorganisms attach to specific receptors on the surface of cells with the help of adhesion molecules in them. They protect themselves against mechanical attacks due to their acid tolerance ability. Thus, the host and pathogen bind adhesins to specific carbohydrate portions of the gastric epithelium. With the attachment that takes place, and inflammatory processes in the gastrointestinal tract begin [5]. Inflammation is the process by which leukocytes and plasma proteins accumulate in tissues and destroy the microorganism. Inflammation begins with the recognition of the microorganism. It can start with innate immune response and continue with an adaptive immune response. Inflammation is divided into acute and chronic. Acute inflammation can take time a few minutes or several hours. Chronic inflammation can vary in ability of body to repair and overcome damage. Complementary system, cytokines, acute phase proteins, and phagocytic cells play a crucial role in inflammation process. The acute inflammatory response is initiated by detecting the presence of the pathogen with pattern recognition receptors, which play an essential role in activating innate immune responses that fight infection [6].

Gastric epithelial cells (GECs) are the foremost target of *H. pylori* bacteria to infect (Figure 1). Toll-like receptors (TLRs) have been adapted to ensure their best response to microbial ligands. It contributes to innate immune responses via signaling. GECs are the primary contact site for *H. pylori.* TLRs activate innate immunity by interacting with extracellular ligands. Even though bacterial toxins are the classic bacterial ligand for TLR4, the bacterial toxin produced from *H. pylori has* been observed to signal via TLR2 and only weakly binds to TLR4 [7]. The permanent existence of the *H. pylori* pathogen is due to multiple virulence factors. In addition to virulence factors, it successfully generates gastrointestinal diseases by manipulating of host immunological responses. Innate immune cells and mucus generated by epithelial cells in the lamina propria are the main defense mechanisms against *H. pylori* [8].

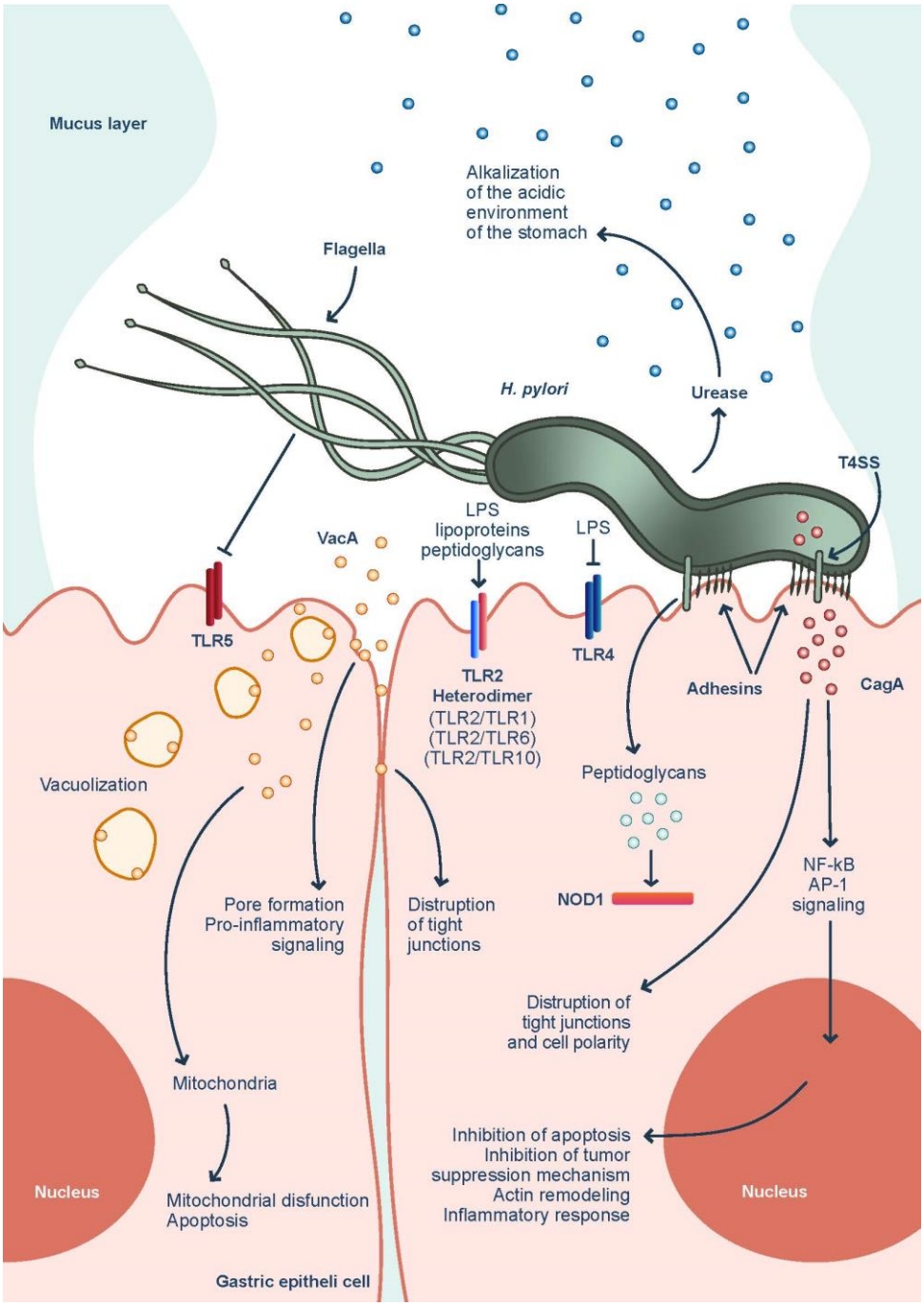

Figure 1. Schematic representation of innate immune response against *H. pylori. H. pylori* can act on GEC through different virulence factors including VacA, CagA, urase, and so on. CagA transportation into the GEC is facilitated by T4SS that *H. pylori use* once it attaches to the cell with several adhesion molecules. CagA could affect cellular responses through the NF-kB and AP-1 pathways which may result in the induction of tumorigenesis through inhibition of apoptosis and tumor suppression mechanisms, actin remodeling, and induction of inflammatory responses. Also, CagA could cause disruption of tight junctions and cell polarity using several mechanisms in the cytosol. Another virulence factor VacA is released into the gastric lumen and acts on the cells by generating pores on the cell membrane, causing vacuolization, mitochondrial disfunction-dependent apoptosis and disruption of tight junctions between GECs. *H. pylori* mediates immune escape by preventing TLR5 recognition of flagella and TLR4 recognition of bacterial LPS. Bacterial LPS, lipoproteins, and peptidoglycans can be recognized by TLR2 heterodimers found on the cell membrane. Also, detection of transported bacterial peptidoglycans into the cytosol of GECs can be facilitated by NOD1 receptors found in the GECs. VacA: Vacuolating toxin A; CagA: Cytotoxin-associated gene A; TLR5: Toll-like receptor 5; TLR4: Toll-like receptor 5; LPS: Lipopolysaccharide; GECs: Gastric epithelial cells; T4SS; Type 4 secretion system.

Innate immunity, which we can call the first line of defense, constitutes the body's first protective barrier against infection. TLRs that interact with extracellular ligands are found in the plasma membrane. They are the main pattern recognition receptor (PRR) clusters that recognize pathogen-associated molecular patterns (PAMPs). The main targets of TLRs with different specificity repertoires are DNA regions rich in bacterial lipopolysaccharides (LPS), peptidoglycan, lipoprotein, lipoteichoic acid and unmethylated CpG. The specific signaling pathways activated by the TLR dimer are determined by the protein adapter that binds to the cytoplasmic TIR (Toll / IL-1 receptor) domain of the TLR. Subsequently, nuclear factor-kappa β (NF-κβ), interferon regulatory factor (IRF), and activator protein-1 (AP-1) is activated. Both NF-κβ and AP-1 are key to the innate immune response, as well as antimicrobial proteins and peptides. It is necessary to trigger the production of IFN-α and IFN-β, as well as proinflammatory cytokines and chemokines [9]. However, TLRs expressed by dendritic cells induce immune responses against *H. pylori.* TLRs recognize the pathogen by binding to a wide variety of protected PAMPs by bacteria. However, *H. pylori* avoids detection [10]. It is thought that *H. pylori* bacteria easily avoid TLRs and hinder recognition due to the low potency of flagellin and lipopolysaccharides (LPS). Particularly, TLR5 specifically detects flagellins in a variety of bacterial pathogens. *H. pylori* has developed mutations in the flagellin to avoid detection by TLR5. The pilus-associated protein CagY, a core component of the type IV secretion system (T4SS) and an essential VirB10 ortholog, encoded by the carcinogenic strains of *H. pylori,* directs effector molecule translocation. In a recent study, CagY was identified as a TLR5 ligand independent of flagellin. Regions were found that allowed CagY positive *H. pylori to* bind to TLR5 expressing cells. Five TLR5 interaction sites have been identified that support TLR5 stimulation and intracellular signal transduction [11]. As a result, CagY is an important VirB10 member detected by TLR5. This interaction can activate innate immune responses by the human pathogen. In another study, flagellins from bacteria are detected by TLR5 in the host, activating an innate immune response. Therefore, efforts have been made to develop vaccines against various infections by fusion with protein antigens. It was designed as a TLR5 agonist by fusion with Bacillus subtilis flagellin, which activates TLR5. Moreover, based on comparative sequence and mutation analyzes, it was revealed that *H. pylori* flagellin (hFIg) was altered to prevent TLR5 recognition by changing residues corresponding to a TLR5 activating hotspot [12].

Some studies have shown that TLRs polymorphisms are related with gastropathies. TLR2 is the best described in the TLR family and it provides production of various pro-inflammatory cytokines by recognizing bacterial LPS, lipoproteins and peptidoglycan. In a study, it was thought that *H. pylori* was associated with gastric cancer and reported increased expression of TLR2 in the gastric mucosa [13]. In one study, it was observed that LPS of some *H. pylori* strains could antagonize TLR4. Therefore, the response of TLR4 to *H. pylori* strains on gastric epithelial cells is low [14]. Flagellin, an essential bacterial structure for motility and colonization, inhibits its recognition by TLR5 by inhibiting the activation of proinflammatory transcription factor and nuclear factor-kappa β (NF-κβ). This is due to the change in the N-terminal D1 domain of the *H. pylori* flagellin, which is crucial for TLR5 recognition [15].

Some of antimicrobial peptides are generated by epithelial cells, a component of the innate immune system, to defend the gastrointestinal epithelium against bacterial invasion. Recent research has demonstrated that *H. pylori can* manipulate these antimicrobial compounds expression to escape from the gastric mucosa and survive there. Antimicrobial peptides (AMPs) are significant members of the innate immune system against *H. pylori.* Peptides then enter the microbes, suppressing DNA, RNA, or protein synthesis. It can have other toxic effects, such as activating antimicrobial enzymes, and can cause cell death. During *H. pylori* infections, AMPs expression can destroy bacteria. Thus, it prevents *H. pylori* infections in the gastrointestinal system. AMPs are generally cysteine-rich, cationic, and amphipathic (comprising both hydrophilic and hydrophobic regions). They are divided into three main groups as α-, β- and θ-defensins. The major source of defensins are human neutrophil peptide-1 (HNP-1), HNP-2 and HNP-3 neutrophils, whereas human defensin 5 (HD-5) and human defensin 6 (HD-6) are expressed by intestinal Paneth cells. Human beta defensin 1 (HBD-1), human beta defensin 2 (HBD-2), and human beta defensin 3 (HBD-3) are plenteously expressed by multiple epithelial cell types, including both in vitro and in vivo GECs. In one study, defensin HBD-1 and inducible HBD-2 and HBD-4 showed a potent antibacterial effect against E. coli. However, it had no effect on *H. pylori.* Binding experiments showed that although HBD-2 did not appear to have a significant adverse effect on the viability of the bacteria, all four *H. pylori* strains tested accumulated on the cell surface. The interaction of HBD-2 to bacteria cause to partial structural changes. However, there was no effective killing, only minor growth inhibition specific to the strain of *H. pylori* was achieved [16]. It belongs a large family of cytosolic proteins which are activated by node-like receptors (NLRs), intracellular pathogen-associated molecular patterns (PAMPs) and substances that stimulate cells against damage or danger (DAMPs and other harmful substances). NLR proteins are divided into two main groups largely

depending on their domain structure. Nucleotide binding oligomerization domain containing protein 1 (NOD1) is associated with endosomes to which it can bind PAMPs, such as diaminopimelic acid (DAP, part of cell peptidoglycans) from cytosolic or endocytosed bacteria. NOD1 dimers activate enzymes that interact with the receptor that initiate NF- κβ signaling. The NOD1 signal reasons the killing of *H. pylori* in the activated epithelial cell via peptide-defensin 2, an antimicrobial peptide. Overall, *H. pylori* has been reported to be recognized by NOD1 in epithelial cells and by NOD2 in DCs produced from bone marrow. Recent studies have not observed that NOD1 blocking does not affect NK-κβ translocation [17]. For mucosal host defense, an epithelial barrier against *H. pylori* infection, NOD1 activation is necessary. This protective effect leads in part to the activation of the type I IFN signaling pathways following the molecular interaction between NOD1 and TRAF3. In one study, they discussed the clinical significance of the interaction between NOD1-TRAF3 in human *H. pylori* associated diseases. Consequently, these studies using human stomach cancer samples have demonstrated that impaired activation of NOD1 and TRAF3 contribute to gastric cancer. At the same time, it has been shown that NOD1-TRAF3 interaction may prevent gastric cancer development [18]. NOD1 activates the type I IFN signaling pathway and promotes mucosal host defense against *H. pylori* infection by production of antimicrobial peptides. Consequently, NOD1-mediated signaling molecules may offer novel therapeutic targets for gastric cancer and chronic gastrointestinal disorders.

H. pylori and Adaptive Immunity

Although the powerful immune responses against *H. pylori,* which can survive for many years by colonizing the human stomach, provide protection for the host, the developing inflammation may cause damage to the host. As serious gastrointestinal diseases may develop in patients; some extraintestinal findings including hematological, cardiovascular, dermatological, and neurological may also occur [19]. Therefore, researching adaptive immune response mechanisms in addition to innate immunity is of great importance in terms of both elucidating the pathogenesis of the disease and targeted treatment approaches [20]. As a result of *H. pylori* colonization, dendritic cells (DCs), macrophages, neutrophils and T and B lymphocytes accumulate in the stomach and a strong and complex inflammatory response is generated by the host (Figure 2) [21]. Studies have shown that CD4+ T helper cells, especially Th1 cells, which differentiate into different subgroups on the effect of cytokines secreted in the adaptive immune response generated by the host, have a greater role than thought [22]. Membrane proteins of *H. pylori* such as Omp18 and HpaA provide maturation and activation of DC, an important APC. DCs that secrete IL-12 cytokine with activation direct T cell-mediated responses to the Th1 type [23]. The neutrophil activating protein (HP-NAP) of *H. pylori* provides the secretion of IL-12 by activating neutrophils and IL-23 by activating monocytes. Thus, it directs Th1 and Th17 polarization in cell-mediated immune responses against *H. pylori* [24]. The activation of Th1 and Th17 cells also provides the secretion of IL-17, IFN-γ and TNF-α. In addition to IFN-γ and TNF-α cytokines also play a major role in the development of *H. pylori* specific Th1 cells [25]. Macrophages activated by *H. pylori* infection stimulate the differentiation of naive CD4+ T cells into the Th17 lymphocyte subtype with the cytokines they secrete IL-6, IL-23 [26]. The virulence factor cytotoxin-associated gene A (CagA) is another factor affecting Th17 differentiation, in which STAT3 and NF-κβ pathways participate [27,28]. Th1 and Th17 cells specific to *H. pylori* that are activated help to eliminate bacteria; however, bacteria that escape from these two cell-mediated immune responses are what lead to gastritis and peptic ulcers because of their abnormally high levels of activation [28]. Although Th2 is effective in the response to extracellular bacteria and helminths, its protective role in the immune response developed against *H. pylori* is still controversial [20,29]. In an experiment with mice, increase in the number of bacteria was found as a result of IL-4 deficiency, and it was observed that the specific Th2 response both reduced bacterial colony and prevented infection [30]. In contrast, distinct study has shown that the Th2 response is not necessary to protect, and the protection is not dependent on antibodies in the absence of IL-4 [31].

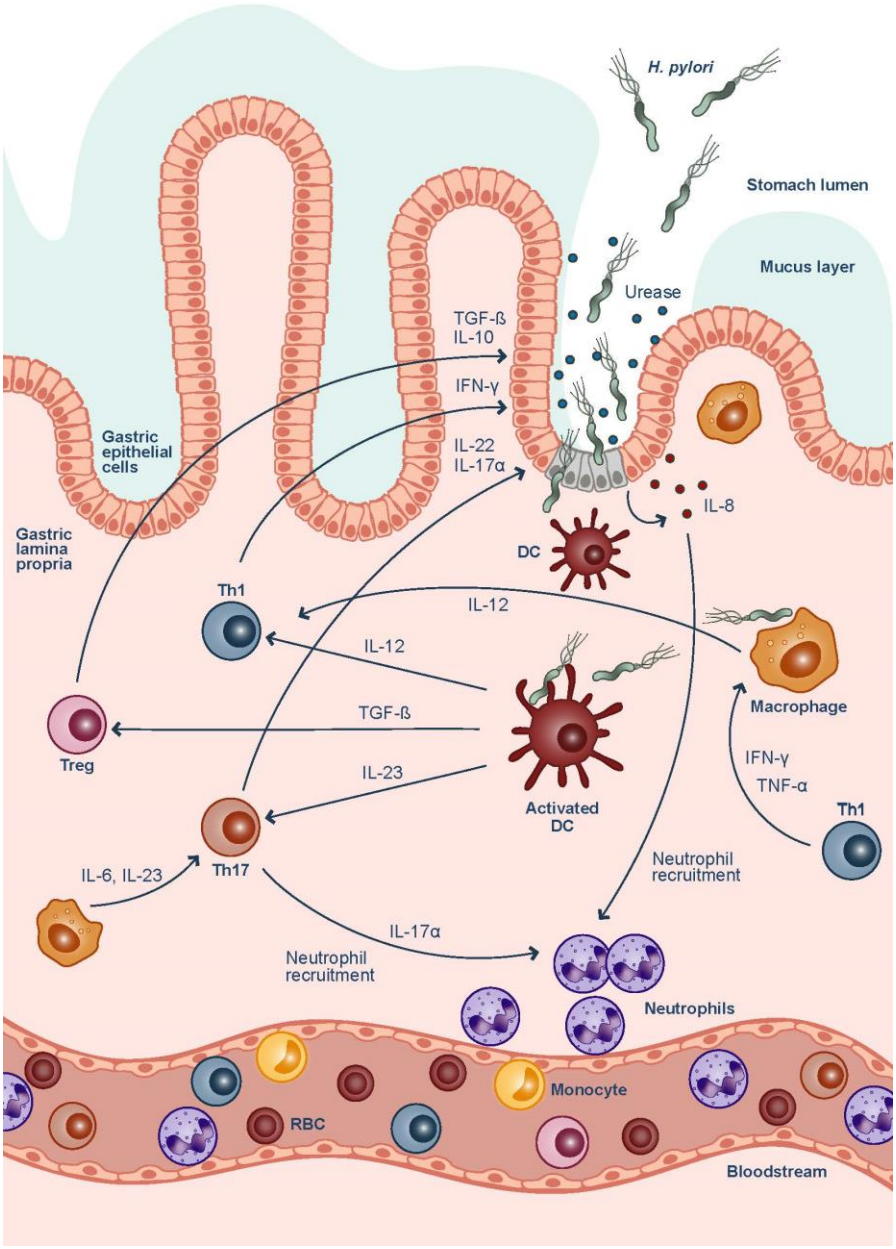

Figure 2. Schematic representation of immune response against *H. pylori infection* in the gastric microenvironment. Immune recognition of *H. pylori starts* when *H. pylori* cross the mucus layer bridge by using the urease enzyme to reach the gastric epithelium. Once epithelial cells are infected by *H. pylori,* pro-inflammatory cytokines such as IL-8 are released which recruits more immune cells to the site of infection. Dendritic cells employ as an important line of defense through phagocytosis of the bacteria to process *H. pylori* particles in order to provide T cell differentiation into Th1, Th17, and Treg cells. Monocytes are recruited to the site of infection where they are differentiated into macrophages, engulf the bacteria and release cytokines such as IL-6, IL-12, and IL-23 to induce the immune system further. Th1 cells that are activated by IL-12 express IFN-γ and TNF-α for proper cleansing of *H. pylori* by macrophages. Also, IFN-γ expressed by Th1 cells activates GECs and triggers apoptosis. Th17 cell polarization provides increasing recruitment of neutrophils by secretion of IL-17α and generation of antimicrobial proteins from GEC through IL-17α and IL-22 activity. Macrophages also increase Th17 polarization in the gastric lamina propria by IL-6 and IL-23. CD4+ T cells are further differentiated into Treg cells to balance immune response and prevent tissue damage because of overstimulation of Th1, Th17, and macrophages in the microenvironment. In the later of the infection, continuous Treg activity may affect GEC to form tumorigenesis by the activity of TGF-β and IL-10. GECs: Gastric epithelial cells; IFN-γ: Interferon γ; IL-6: Interleukin 6; TGF-β: Transforming growth factor β; Th1: T helper 1 cell; Th17: T helper 17 cell; Treg: T regulatory cell; TNF-α: Tumor necrosis factor α.

Treg cells, which have a main role in regulating immune response and maintaining peripheral tolerance, are also of importance in the balance of mucosal immune response in the stomach against *H. pylori* infection [20,28]. Studies have shown that in *H. pylori* infected individuals, CD4+CD25 (high) Treg cells expressing FOXP3 mRNA and cytokines IL-10 and TGFβ are highly expressed [32]. At the same time, an increase in CTLA-4 protein, which is a protein that suppresses the immune response, was observed [10]. Inhibition of CD4+ T cell proliferation by Treg cell development can protect host cells against excessive gastric ulcer immunopathology, while supporting bacterial colonization and may provide gastric tumor development [7,22,28,30]. It is necessary to consider the role of not only cellular immunity but also humoral immunity against *H. pylori* infection. In one study, increased expression of CXCL13, which regulates B cell homing in the gastric mucosa of individuals infected with *H. pylori* and CXCR5, the receptor of CXCL3 in CD20+ lymphocyte aggregates, was observed [33]. Another study has been shown that a ligand APRIL that induces B cell proliferation contributes to the development of *H. pylori*-induced gastric MALT lymphoma [34]. In the research, the presence of mucosal and local IgA and IgG antibodies was found to be high in *H. pylori* infected individuals. However, the effect of these antibodies on bacterial colonization is not clear. On the other hand, it should not be ignored that B cells may cause the development of autoimmunity in *H. pylori*-infected individuals. Depending on the infection, autoantibodies can be produced that can cross-react with H+K+-ATPase in parietal cells. As a result, the host stomach is damaged due to complement activation, apoptosis or ADCC triggered by these autoantibodies and inflammation develops [10,35].

H. pylori Genetics and Vaccination

The seriousness of diseases caused by *H. pylori* is linked to several virulence factors, one of which is the genotype of the *H. pylori* strain. Moreover, a dynamic relationship within the host, the gastrointestinal microenvironment, and virulence factors of bacteria are important. Inflammatory responses are not only induced by *H. pylori* virulence agents, but they are also controlled and regulated by them, resulting in chronic inflammation. *H. pylori* colonization and survival inside the gastric mucosa are promoted by virulence factors, allowing for more immune evade and, eventually, the introduction of premalignant changes. *H. pylori* has a broader range of functions that influence the host's cellular responses and signaling pathways [8]. Whole-genome sequencing was conducted on multiple strains of *H. pylori,* and Outer membrane proteins (OMPs) contained about 4% of their genes. Similar to their roles, five paralogous gene families are described here. The most well-known families of proteins are Hop (outer membrane porins) and Hor (Hop-related proteins). Hof (*H. pylori* OMP) and Hom (*H. pylori* outer membrane) are the second and third proteins, respectively. The fourth family of OMPs is iron-regulated, and the fifth family is efflux pump OMPs. The alleles, geographic distribution features, and associations with several other virulence factors of OMPs which do not relate to either of these families that all are unique. The most popular strategies for controlling OMP expression are allelic variation and phase variation. Each OMP generally has several alleles, and the proteins encoded from each allele have slightly different functions [36]. OMPs participate in colonization of *H. pylori.* Furthermore, *H. pylori* adherence to gastric epithelial cells is a multi-step mechanism containing target receptors and several adhesins. Besides different OMPs may respond to environmental changes by rapidly controlling themselves in return for alterations in gastric inflammation and pH. OMPs not only facilitate bacterial attachment to epithelial cells of the gastric mucosa, but they also collaborate with some of the virulence factors, including CagA and VacA to raise the production of inflammatory factors, resulting in a range of patient outcomes [37]. *H. pylori* strains perform a variety of functions in the development of GC. Bacterial oncoprotein CagA positive and negative strains of *H. pylori* can be distinguished. People who are infected with CagA positive strains have a greater possibility for developing GC, according to a meta-analysis, which is like prior findings that people with CagA antibodies have an increased chance of cancer. The process, on the other hand, seems to be somewhat complicated. Through the induction of integrin, *H. pylori* injects CagA into the recipient gastric epithelial cells [37,38]. CagA is also phosphorylated on tyrosine by Src family kinases or Abl kinase, which stimulates various signaling pathways. Phosphorylated CagA, for example, is associated with triggered SHP2. CagA-SHP2 increases the frequency of Erk-MAP kinase activation at either Ras-dependent or Ras-independent forms [39]. Nonphosphorylated CagA often degrades intracellular signaling pathways. The nonphosphorylated intracellular CagA communicates with E-cadherin, causing the E-cadherin–-catenin complex to be disrupted. Therefore, nuclear -catenin accumulates, facilitating transcription of carcinogenesis-related gene expression. CagA, on the other hand, has been shown to trigger-catenin directly by engaging with MET and triggering PI3K–AKT signaling [40]. The signal transducer and activator of transcription 3 (STAT3) pathway is induced by CagA. Free of CagA phosphorylation, the immune response of the host stimulates the STAT3 pathway, which is related to *H. pylori*-stimulated gastritis and cancer development [27]. Along with CagA, the *H. pylori* peptidoglycan is also delivered into host cells by the Cag

secretory mechanism via outer membrane vesicles. The PI3K-AKT pathway, which regulates cellular proliferation, migration, and death, is then triggered by the peptidoglycan. Apart from Cag, another essential virulence predictor of *H. pylori* is vacuolating toxin A (VacA). The excreted protein VacA is encoded by the *H. pylori* gene VacA. Multiple cellular mechanisms have been related to VacA, including vacuolation, membrane-channel development, apoptosis, proinflammatory reaction, and tumor [41].

Clarithromycin (CLA) has been the cornerstone of *H. pylori* therapy because it has lower minimal inhibitory concentration, efficient mucosal diffusion, and very minor effect on gastric acid secretion. This treatment includes triple therapy for a considerable period, which included CLA, amoxicillin or metronidazole, and a proton pump inhibitor (PPI). Although, CLA has regressed effect of triple therapy because of the resistant *H. pylori* and contributes to the increase of multidrug loading in gram-negative infections. Point mutations in the peptidyl transferase loop of the 23S rRNA gene's V domain have been linked to the CLA resistance phenotype in clinical *H. pylori* strains from various geographical locations, according to certain PCR investigations. These alterations might interfere with the peptidyl transferase loop structure and lessen its efficiency by preventing the binding of CLA to 23S rRNA. It can also cause a resistance phenotype [42].

In 2017, the World Health Organization identified *H. pylori,* which is highly resistant to clarithromycin, as a priority in antibiotic research and development studies. In some countries, it is seen that the antimicrobial eradication rate falls below 60%. It is thought that long-term treatment with multiple antibiotics may increase resistance even more [43]. Moreover, reducing the use of antibiotics against antibiotic resistance that may occur in the coming years, also new strategies are needed to eradicate *H. pylori.* Designing an effective vaccine against *H. pylori* infection is the ideal therapeutic way. Since the discovery of the bacterium, various vaccine studies have been carried out in animal studies that have not been successful in eradicating the infection completely in humans at an intermediate level [44]. Making a vaccination is technically challenging since *H. pylori* lives in the gastric mucosa. *H. pylori,* which is strongly linked to the GEC surface and stomach mucosa, isn't typically highly invasive. Consequently, majority of immunological effector mechanisms might be excluded. Neutrophils accumulate in the intestinal mucosa and cross the epithelium to create crypt abscesses. At the same time, although it contains antibodies and antimicrobial peptides secreted in the gastric mucosa, these mechanisms are not sufficient to eradicate *H. pylori* infection [45].

Numerous investigations have been carried out in recent years to locate bacterial antigens and epitopes which can aid the generation of a vaccine that can eliminate the *H. pylori* infection. Multiple *H. pylori* proteins, including Urease, BabA, VacA, Hsp60, HpaA, NAP, and CagA, have been implicated as effective vaccine antigens. It has been demonstrated that using these antigens in combination with an effective adjuvant decreases *H. pylori* colonization [46].

The multivalent epitope based CWAE vaccine uses proteins like Hsp60, NAP, urease, and HpaA to protect against *H. pylori.* In the Mongolian gerbil model, it has been observed that the CWAE vaccination has a remarkable therapeutic efficacy. IgG antibodies produced by CWAE have been demonstrated to limit *H. pylori urease* activity in a dose-dependent approach. A better inhibitory effect of specific IgG was observed in Mongolian gerbils vaccinated with CWAE [47].

Guo and coauthors observed that CFdAE vaccine combined with a polysaccharide adjuvant (PA) significantly diminished *H. pylori* abundance in the stomach of mice compared with urease. Histologically, mice vaccinated with CFdAE vaccine showed weaker inflammation in the stomach than the Mongolian gerbil model inoculated with urease + PA [48].

Zhou and coauthors The HUepi-LTB vaccine he designed is a multi-epitope oral vaccine which contains two B cell and three Th cell epitopes from UreB and HpaA. It has been observed that the HUepi-LTB vaccination considerably lowers the bacterial burden in the stomach of mice. Following the H. uepi-LTB vaccination, there was a considerable increase in the specific IgG, IgG1 and IgG2a levels against *H. pylori lysates*. Also, therapeutic immunization with HUepi-LTB vaccine was observed to induce greater secretion of mucosal IgA and serum IgG specific to *H. pylori.* In this case, specific humoral immune responses have also been associated with lower *H. pylori* colonization [49].

In a different investigation, mice were used to assess the effectiveness of an oral probiotic vaccine made from Lactococcus lactis that expresses UreB. The test's results, it was found to induce anti-urease antibody responses and reduce colonization of *H. pylori* in stomach. UreB subunit is known as the most effective immunogen among *H. pylori* strains. More anti-UreB antibodies and more cytokines that particularly bind to the bacterial UreB protein were secreted when mice were immunized with recombinant Lactococcus lactis expressing the UreB-IL-2 protein [50].

In the oral recombinant vaccine developed in China, the urease B subunit fused with the heat-sensitive enterotoxin B subunit was used. The efficacy of the developed three-dose oral recombinant vaccine was

tested in children. In conclusion, oral recombinant *H. pylori* vaccine was found to be more effective in children who did not receive *H. pylori* before [51].

Aebischer and coauthors investigated live vaccines based on the Typhoid vaccine, recombinant Salmonella Ty21a, in volunteers with the presence of *H. pylori.* T cell reactivity to *H. pylori* antigens has been observed to significantly reduce the *H. pylori* burden [52].

A vaccine consisting of three recombinant Helicobacter pylori antigens (VacA), cytotoxin-associated antigen (CagA) and neutrophil activating protein (NAP) was tested in an animal model and appeared to prevent infection. The vaccine demonstrated the existence of cell-mediated immune responses and long-term T-cell memory. The efficacy of the vaccine was investigated in healthy volunteers orally vaccinated with the CagA-positive *H. pylori* strain. The vaccine did not provide any protection against *H. pylori* infection after challenge with a CagA-positive strain [53]. Research on the creation of anti-*H. pylori* vaccines has led to a better understanding of the interaction between the pathogen and the host's immune system.

CONCLUSION

Developing a reliable vaccination has been limited by the inconsistent study findings and the discontinuity of clinical trials. With rising challenges in infection eradication therapies and high infection rates globally, it is evident that additional research is needed to determine a viable treatment against *H. pylori.* The clinical symptoms that may occur because of *H. pylori* infection are connected to the virulence factors of the bacteria, the genetic factors of the host and the immune response. Understanding adaptive immune response mechanisms as well as innate immunity has an indispensable place in terms of both elucidating the pathogenesis of the disease and developing new targeted therapy approaches.

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REFERENCES

1. Robin Warren J, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. 1983 Jun 4;321(8336):1273–5.
2. Morris A, Nicholson G. Ingestion of Campylobacter pyloridis causes gastritis and raised fasting gastric pH. Am J Gastroenterol. 1987;82(3):192–9.
3. Bilgehan H. Clinical Microbiology Special Bacteriology and Bacterial Infections. Microbiology. İstanbul: Barıs Press; 1990.
4. Murray PR, Rosenthal KS, Michael A. Pfaller. Medical Microbiology 9th Edition. Angew Chemie Int Ed 6(11), 951–952. 1967;5–24.
5. Sit WY, Chen YA, Chen YL, Lai CH, Wang WC. Cellular evasion strategies of Helicobacter pylori in regulating its intracellular fate. Semin Cell Dev Biol. 2020 May 1;101:59.
6. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med 2019 2512. 2019 Dec 5;25(12):1822–32.
7. Walduck A, Andersen LP, Raghavan S. Inflammation, Immunity, and Vaccines for Helicobacter pylori Infection. Helicobacter. 2015 Sep 1;20:17–25.
8. Baj J, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, et al. Helicobacter pylori Virulence Factors-Mechanisms of Bacterial Pathogenicity in the Gastric Microenvironment. Cells. 2020 Jan 1;10(1):1–37.
9. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol. 2014;5(SEP):461.
10. Peek RM, Fiske C, Wilson KT. Role of Innate Immunity in Helicobacter pylori-Induced Gastric Malignancy. Physiol Rev. 2010 Jul;90(3):831.
11. Tegtmeyer N, Neddermann M, Lind J, Pachathundikandi SK, Sharafutdinov I, Gutiérrez-Escobar AJ, et al. Toll-like Receptor 5 Activation by the CagY Repeat Domains of Helicobacter pylori. Cell Rep. 2020 Sep 15;32(11):108159.
12. Kim JH, Namgung B, Jeon YJ, Song WS, Lee J, Kang SG, et al. Helicobacter pylori flagellin: TLR5 evasion and fusion-based conversion into a TLR5 agonist. Biochem Biophys Res Commun. 2018 Nov 2;505(3):872–8.
13. Uno K, Kato K, Atsumi T, Suzuki T, Yoshitake J, Morita H, et al. Toll-like receptor (TLR) 2 induced through TLR4 signaling initiated by Helicobacter pylori cooperatively amplifies iNOS induction in gastric epithelial cells. Am J Physiol - Gastrointest Liver Physiol. 2007 Nov;293(5):1004–12.
14. Gong Y, Tao L, Jing L, Liu D, Hu S, Liu W, et al. Association of TLR4 and Treg in Helicobacter pylori Colonization and Inflammation in Mice. PLoS One. 2016 Feb 1;11(2):e0149629.

15. Pachathundikandi SK, Tegtmeyer N, Arnold IC, Lind J, Neddermann M, Falkeis-Veits C, et al. T4SS-dependent TLR5 activation by Helicobacter pylori infection. Nat Commun 2019 101. 2019 Dec 16;10(1):1–11.
16. Pero R, Coretti L, Nigro E, Lembo F, Laneri S, Lombardo B, et al. β-Defensins in the Fight against Helicobacter pylori. Mol A J Synth Chem Nat Prod Chem. 2017 Mar 1;22(3).
17. Minaga K, Watanabe T, Kamata K, Asano N, Kudo M. Nucleotide-binding oligomerization domain 1 and Helicobacter pylori infection: A review. World J Gastroenterol. 2018 Apr 4;24(16):1725.
18. Suarez G, Romero-Gallo J, Piazuelo MB, Wang G, Maier RJ, Forsberg LS, et al. Modification of Helicobacter pylori Peptidoglycan Enhances NOD1 Activation and Promotes Cancer of the Stomach. Cancer Res. 2015 Apr 15;75(8):1749–59.
19. Ražuka-Ebela D, Giupponi B, Franceschi F. Helicobacter pylori and extragastric diseases. Vol. 23, Helicobacter. 2018.
20. Algood HMS, Cover TL. Helicobacter pylori persistence: An overview of interactions between H. pylori and host immune defenses. Vol. 19, Clinical Microbiology Reviews. 2006.
21. Razavi A, Bagheri N, Azadegan-Dehkordi F, Shirzad M, Rahimian G, Rafieian-Kopaei M, et al. Comparative Immune Response in Children and Adults with H. pylori Infection. Vol. 2015, J Immunol Res. 2015.
22. Moyat M, Velin D. Immune responses to Helicobacter pylori infection. World J Gastroenterol. 2014 May 5;20(19):5583.
23. Akhiani AA, Pappo J, Kabok Z, Schön K, Gao W, Franzén LE, et al. Protection against Helicobacter pylori infection following immunization is IL-12-dependent and mediated by Th1 cells. J Immunol. 2002 Dec 15;169(12):6977–84.
24. Amedei A, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, et al. The neutrophil-activating protein of Helicobacter pylori promotes Th1 immune responses. J Clin Invest. 2006 Apr 4;116(4):1092.
25. Goll R, Gruber F, Olsen T, Cui G, Raschpichler G, Buset M, et al. Helicobacter pylori stimulates a mixed adaptive immune response with a strong T-regulatory component in human gastric mucosa. Helicobacter. 2007 Jun;12(3):185–92.
26. Zhuang Y, Shi Y, Liu XF, Zhang JY, Liu T, Fan X, et al. Helicobacter pylori-infected macrophages induce Th17 cell differentiation. Immunobiology. 2011 Jan;216(1–2):200–7.
27. Menheniott TR, Judd LM, Giraud AS. STAT3: a critical component in the response to Helicobacter pylori infection. Cell Microbiol. 2015 Nov 1;17(11):1570–82.
28. Jafarzadeh A, Larussa T, Nemati M, Jalapour S. T cell subsets play an important role in the determination of the clinical outcome of Helicobacter pylori infection. Microb Pathog. 2018 Mar 1;116:227–36.
29. Larussa T, Leone I, Suraci E, Imeneo M, Luzza F. Helicobacter pylori and T Helper Cells: Mechanisms of Immune Escape and Tolerance. J Immunol Res. 2015;2015.
30. Mohammadi M, Nedrud J, Redline R, Lycke N, Czinn SJ. Murine CD4 T-cell response to Helicobacter infection: TH1 cells enhance gastritis and TH2 cells reduce bacterial load. Gastroenterology. 1997;113(6):1848–57.
31. Garhart CA, Nedrud JG, Heinzel FP, Sigmund NE, Czinn SJ. Vaccine-Induced Protection against Helicobacter pylori in Mice Lacking Both Antibodies and Interleukin-4. Infect Immun. 2003 Jun 1;71(6):3628.
32. Lundgren A, Strömberg E, Sjöling Å, Lindholm C, Enarsson K, Edebo A, et al. Mucosal FOXP3-Expressing CD4+ CD25high Regulatory T Cells in Helicobacter pylori-Infected Patients. Infect Immun. 2005 Jan;73(1):523.
33. Nakashima Y, Isomoto H, Matsushima K, Yoshida A, Nakayama T, Nakayama M, et al. Enhanced expression of CXCL13 in human Helicobacter pylori-associated gastritis. Dig Dis Sci. 2011 Oct;56(10):2887–94.
34. Munari F, Lonardi S, Cassatella MA, Doglioni C, Cangi MG, Amedei A, et al. Tumor-associated macrophages as major source of APRIL in gastric MALT lymphoma. Blood. 2011 Jun 16;117(24):6612–6.
35. Robinson K, Argent RH, Atherton JC. The inflammatory and immune response to Helicobacter pylori infection. Best Pract Res Clin Gastroenterol. 2007;21(2).
36. Xu C, Soyfoo DM, Wu Y, Xu S. Virulence of Helicobacter pylori outer membrane proteins: an updated review. Eur J Clin Microbiol Infect Dis. 2020 Oct 1;39(10):1821–30.
37. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-Analysis of the Relationship between cagA Seropositivity and Gastric Cancer. Gastroenterology. 2003;125(6):1636–44.
38. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40(3):297.
39. Higashi H, Nakaya A, Tsutstumi R, Yokoyama K, Fujii Y, Ishikawa S, et al. Helicobacter pylori CagA Induces Ras-independent Morphogenetic Response through SHP-2 Recruitment and Activation. J Biol Chem. 2004;279(17).
40. Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, et al. Helicobacter pylori CagA Phosphorylation-Independent Function in Epithelial Proliferation and Inflammation. Cell Host Microbe. 2009;5(1).
41. Yamaoka Y. Mechanisms of disease: Helicobacter pylori virulence factors. Vol. 7, Nature Reviews Gastroenterology and Hepatology. 2010.
42. Marques AT, Vítor JMB, Santos A, Oleastro M, Vale FF. Trends in helicobacter pylori resistance to clarithromycin: From phenotypic to genomic approaches. Vol. 6, Microbial Genomics. 2020.
43. Suzuki S, Esaki M, Kusano C, Ikehara H, Gotoda T. Development of Helicobacter pylori treatment: How do we manage antimicrobial resistance? World J Gastroenterol. 2019;25(16).
44. Meyer TF, Morey P. A future for a vaccine against the cancer-inducing bacterium helicobacter pylori? In: Mucosal Vaccines: Innovation for Preventing Infectious Diseases. 2019.

45. Ikuse T, Blanchard TG, Czinn SJ. Inflammation, immunity, and vaccine development for the gastric pathogen helicobacter pylori. In: Current Topics in Microbiology and Immunology. 2019.
46. Chen J, Lin L, Li N, She F. Enhancement of Helicobacter pylori outer inflammatory protein DNA vaccine efficacy by co-delivery of interleukin-2 and B subunit heat-labile toxin gene encoded plasmids. Microbiol Immunol. 2012 Feb;56(2):85–92.
47. Guo L, Yang H, Tang F, Yin R, Liu H, Gong X, et al. Oral Immunization with a multivalent epitope-based vaccine, based on NAP, Urease, HSP60, and HpaA, provides therapeutic effect on H. pylori infection in Mongolian gerbils. Front Cell Infect Microbiol. 2017;7(AUG).
48. Guo L, Yin R, Xu G, Gong X, Chang Z, Hong D, et al. Immunologic properties and therapeutic efficacy of a multivalent epitope-based vaccine against four Helicobacter pylori adhesins (urease, Lpp20, HpaA, and CagL) in Mongolian gerbils. Helicobacter. 2017;22(6).
49. Zhou WY, Shi Y, Wu C, Zhang WJ, Mao XH, Guo G, et al. Therapeutic efficacy of a multi-epitope vaccine against Helicobacter pylori infection in BALB/c mice model. Vaccine. 2009;27(36).
50. Zhang H xin, Qiu Y yu, Zhao Y hui, Liu X ting, Liu M, Yu A lian. Immunogenicity of oral vaccination with Lactococcus lactis derived vaccine candidate antigen (UreB) of Helicobacter pylori fused with the human interleukin 2 as adjuvant. Mol Cell Probes. 2014;28(1).
51. Zeng M, Mao XH, Li JX, Tong W De, Wang B, Zhang YJ, et al. Efficacy, safety, and immunogenicity of an oral recombinant Helicobacter pylori vaccine in children in China: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2015;386(10002).
52. Aebischer T, Bumann D, Epple HJ, Metzger W, Schneider T, Cherepnev G, et al. Correlation of T cell response and bacterial clearance in human volunteers challenged with Helicobacter pylori revealed by randomised controlled vaccination with Ty21a-based Salmonella vaccines. Gut. 2008 Aug;57(8):1065–72.
53. Malfertheiner P, Selgrad M, Wex T, Romi B, Borgogni E, Spensieri F, et al. Efficacy, immunogenicity, and safety of a parenteral vaccine against Helicobacter pylori in healthy volunteers challenged with a Cag-positive strain: a randomised, placebo-controlled phase 1/2 study. Lancet Gastroenterol Hepatol. 2018 Oct 1;3(10):698–707.