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# *Helicobacter pylori* virulence factors: subversion of host immune system and development of various clinical outcomes

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**Review**

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## Abstract

*Helicobacter pylori* (*H. pylori*) is a worldwide spread bacterium, co-evolving with humans for at least 100 000 years. Despite the uncertainty about the mode of *H. pylori* transmission, the development of intra-gastric and extra-gastric diseases is attributed to this bacterium. The morphological transformation and production of heterogenic virulence factors enable *H. pylori* to overcome the harsh stomach environment. Using numerous potent disease-associated virulence factors makes *H. pylori* a prominent pathogenic bacterium. These bacterial determinants are adhesins (e.g., blood group antigen-binding adhesin (BabA)/sialic acid-binding adhesin (SabA)), enzymes (e.g., urease), toxins (e.g., vacuolating cytotoxin A (VacA)), and effector proteins (e.g., cytotoxin-associated gene A (CagA)) involved in colonisation, immune evasion, and disease induction. *H. pylori* not only cleverly evades the immune system but also robustly induces immune responses. This insidious bacterium employs various strategies to evade human innate and adaptive immune responses, leading to a life-long infection. Owing to the alteration of surface molecules, innate immune receptors couldn't recognise this bacterium; moreover, modulation of effector T cells subverts adaptive immune response. Most of the infected humans are asymptomatic and only a few of them present severe clinical outcomes. Therefore, the identification of virulence factors will pave the way for the prediction of infection severity and the development of an effective vaccine. *H. pylori* virulence factors are hereby comprehensively reviewed and the bacterium evasion from the immune response is properly discussed.

## Introduction

The spiral-shaped, Gram-negative microaerophilic bacterium, *Helicobacter pylori* (*H. pylori*) colonises the mucosal layer of the stomach epithelium of more than 50% of mankind (1). *H. pylori*-human co-existence is ~100 000 years old indicating a great adaptation of *H. pylori* to its niche in the human gastric mucosa (2, 3). Recently, Hooi *et al.* and Zamani *et al.* conducted two separate systematic reviews and meta-analyses on *H. pylori*'s global prevalence (1, 4). A high prevalence of *H. pylori* was reported by the first meta-analysis (60.3%); the lowest prevalence was found in Northern America (37.1%) and Oceania (24.4%) and the highest prevalence in Africa (79.1%), Latin America, and the Caribbean (63.4%), and Asia (54.7%) (1). A lower prevalence was suggested by the second meta-analysis (44.3%), ranging from 34.7% in developed countries to 50.8% in developing countries (4). The presence of the organism in the oral cavity, water and food introduces them as potential routes of *H. pylori* transmission (5, 6). Considering that the oral cavity provides an optimal pH, temperature, and microaerophilic environment, oral–oral (particularly mother-to-child) is the prominent mode of *H. pylori* transmission in both developing and developed countries (7, 8). *H. pylori* dwelling in the oral cavity is assumed as a risk factor for the recrudescence of gastric *H. pylori* infection (9). Recrudescence or re-infection leads to recurrence (9). Therefore, the recurrence of *H. pylori* is considered a critical issue (10). The pathogenicity of *H. pylori* is attributed to several mechanisms including (i) manipulation of the host signalling pathways, (ii) induction of indirect inflammatory responses within the gastric mucosa, and (iii) induction of direct epigenetic changes on gastric epithelial cells (11). Despite inducing an inflammatory response, the human immune system could not eradicate bacterial colonisation, resulting in a life-long infection (12). *H. pylori* is a class-I carcinogen and it is known as a main risk factor for intra-gastric diseases including chronic gastritis (CG), peptic ulcer diseases (PUD), gastric cancer (GC), MALT lymphoma (MALToma) and biliary tract cancer (13-17) as well as extra-gastric diseases, including cardiovascular, metabolic, and neurologic disorders (18). The complex interplay among the three major factors including: (i) bacterial virulence factors (e.g., cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA) and blood group antigen-binding adhesion (BabA), (ii) host genetic traits (e.g., tumour necrosis factor-alpha

(TNF-α), interleukin 10 (IL-10) and IL-1β), and (iii) environmental factors (e.g., high salt, diet, meat consumption and smoke) would result in the diverse clinical outcomes from asymptomatic infection to GC (19-21). Many factors, including geographic regions, living environment, socioeconomic status, educational level, and age are involved in the prevalence of *H. pylori* (22). Gut microbiome, obesity, male gender, consumption of unpasteurised dairy products, and high-risk occupations (e.g., healthcare and sheepherding) predispose individuals to *H. pylori* infection (23-29). The development of various adaptations enables *H. pylori* to effectively colonise in harsh stomach conditions (3). Using various virulence factors, *H. pylori* colonises the stomach mucosa, escapes the immune response, and induces the disease (Table 1) (30). *H. pylori* passes through four steps to colonise, persist and cause serious disease in the human host: (i) overcoming the harsh acidic stomach condition by bacterial urease enzyme, (ii) moving toward epithelium cells by polar flagella and penetrating gastric mucosal barrier, (iii) the interaction of bacterial adhesins (e.g., CagL, CagY, OipA, HopQ, HopZ, BabA/B and SabA/B) with glycan structures (Gly-Rs) exposed on the external surface of gastric epithelial cells and the mucus layer, and (iv) releasing the toxins (e.g., VacA and CagA) causing tissue damages (31). Besides, the virulence factors such as urease, flagellin, arginase and TlpB (a transmembrane chemoreceptor) are survival proteins and the virulence factors including neutrophil-activating protein A (NapA), γ-glutamyl transpeptidase (GGT), peptidoglycan (PG) and ADP-Heptose are immunoresponsive elements (32). Furthermore, morphological transformation (from spiral to coccoid) is an interesting issue that has been recently considered owing to its vital role in the survival of *H. pylori* in the host gastric microenvironment (33, 34). Although *H. pylori* numerous virulence factors are responsible for the induction of local damage in gastric mucosa, the host's innate and adaptive immunity including the pattern recognition receptors (PRRs), pro-inflammatory cytokines, chemokines, chemotactic factors, monocytes, macrophages, neutrophils, natural killer cells (NK cells), dendritic cells (DCs), B cells, and T cells facilitate the development of subclinical systemic inflammation (35-37). Therefore, the presence of *H. pylori* in the host gastric mucosa motivates the host's immune system to trigger systemic damage (38). Despite this strong inflammatory response, *H. pylori*'s ability to evasion, subversion and manipulation of the host immune responses guarantees the development of persistent infection in the stomach mucosa. Alteration in the surface molecules is an efficient strategy that preserves the bacterium from being recognised by the innate immune system (39). Besides, modulation of the function of the T cells by *H. pylori* disrupts the host's adaptive immunity against this bacterium (40). *H. pylori* prevention and eradication are among the challenging issues in the post-antibiotic era; therefore, providing alternative drugs or vaccine

**Table 1.** Virulence factors involving in different aspects of *H. pylori* pathogenesis (colonization, immune escape, and disease induction), their functions, and associated diseases.

| Colonization | | Immune escape | | Disease induction | |
|---|---|---|---|---|---|
| Virulence factors | Biological functions | Virulence factors | Biological functions | Virulence factors | Associated diseases |
| Urease | Neutralize gastric acid | LPS<br>Flagella | Low immunogenicity<br>Molecular mimicry<br>Induce anti-inflammatory response | *cag*PAI<br>CagA | GC<br>CG<br>MALToma<br>PUD |
| Flagella<br>Chemotaxis system | Bacterial movement to epithelial surface and deep gland | CagT4SS<br>CagA | Suppress phagocytosis<br>Decrease antimicrobial peptide<br>Induce tolerogenic dendritic cell<br>Block effector T cell response | VacA | GC<br>PUD |
| Adhesins<br>BabA/B<br>SabA/B<br>AlpA/B<br>HopZ/Q<br>CagL/Y<br>NapA<br>OipA<br>LabA<br>Hsp60 | Adherence to gastric epithelial cells | VacA | Suppress phagocytosis<br>Induce tolerogenic dendritic cell<br>Block effector T cell response | BabA | GC<br>PUD |
| | | GGT | Induce tolerogenic dendritic cell<br>Block effector T cell response | HtrA | GC |
| | | Cholesterol-α-glucosyltransferase | Suppress phagocytosis | DupA | PUD<br>DU<br>Decreased GC |
| | | Catalase<br>Superoxide dismutase | Suppress ROS and NO | IceA | PUD |
| | | Arginase | Suppress ROS and NO<br>Block effector T cell response | OipA | GC<br>PUD<br>DU<br>Dysplasia |

*cag*PAI, *cag* pathogenicity island; CagT4SS, Cag type IV secretion system; CagA, cytotoxin-associated gene A; VacA, vacuolating cytotoxin A; HtrA, high temperature requirement A; DupA, duodenal ulcer promoting gene A; IceA, induced by contact with epithelium gene A; OipA, outer inflammatory protein A; GGT, γ-glutamyl transpeptidase; BabA/B, blood group antigen-binding adhesin A/B; SabA/B, sialic acid-binding adhesin A/B; AlpA/B, adherence-associated proteins A/B; HopZ/Q, *H. pylori* outer membrane protein Z/Q; CagL/Y, cytotoxin associated gene L/Y; NapA, neutrophil-activating protein A; LabA, LacdiNAc-binding adhesin; LPS, lipopolysaccharide; HSP60, heat shock protein 60. ROS, reactive oxygen species; NO, nitric oxide; CG, chronic gastritis; GC, gastric cancer; MALToma, MALT lymphoma; PUD, peptic ulcer diseases; DU, duodenal ulcer.

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targets is an urgent need (41). In this regard, highlighting the importance of bacterial virulence factors in *H. pylori* pathogenesis is a promising strategy. This review provides comprehensive data on the *H. pylori* virulence factors and discusses this bacterium's evasion strategies from the immune response.

## The genome and virulence factors of *H. pylori*

The genome of *H. pylori* was among the first bacterial species which was completely sequenced.

According to the obtained data, *H. pylori*, strain 26 695, possesses a circular genome of 1 667 867 bp and 1590 predicted coding sequences (42, 43). The whole-genome sequencing of *H. pylori* revealed extraordinary genetic flexibility and a high frequency of gene recombination; this unique nature qualifies the bacteria to survive in harsh and dynamic habitats (44, 45). Recognition of virulence factors might pave the way to the illustration of the *H. pylori* pathogenesis and prediction of the risk for inducing intra- and extra-gastric diseases (46).

### *Cytotoxin-associated gene pathogenicity island*

Cytotoxin-associated gene pathogenicity island (*cag*PAI) is existent in nearly 70% of all *H. pylori* strains isolated globally, compared with 60% of western isolates and 95% of East Asian isolates (47, 48). The *cag*PAI has been integrated into the *H. pylori* DNA via horizontal gene transfer (HGT), although its origin is unknown and its genes are not essential for *H. pylori* (49). *cag*PAI is a ~40 kb genomic region containing 32 open reading frames (ORFs), namely *cag1-26*, *cagA-Z* or *cagα-ζ*, or by locus name of the HP 26 695 or HP J99 strain genomes (50). *cag*PAI encode effector protein CagA and type IV secretion system (CagT4SS), a syringe-like structure to inject CagA into gastric epithelial cells (51, 52). Various bacterial molecules including CagA, DNA and PG metabolites are translocated into host cells by CagT4SS (53-55). Backert *et al.* suggested that intact CagT4SS consists of a core complex (CagT, CagX, CagM, Cagδ, and CagY), with associated factors (CagH, CagN, CagU, CagV, and CagW); pilus components (CagC, CagH, CagI, CagL, and CagY); and energetic components (CagE, Cagα, and Cagβ). Besides, translocation-associated factors (CagF, CagZ, and Cagβ), and a lytic trans-glycosylase (Cagγ) exist in CagT4SS (51). Although the complete composition of *cag*PAI guarantees encoding of intact CagT4SS, *cag*PAI is absent in nearly 30% of *H. pylori* strains, and it is incomplete in some strains (49, 56). The severity of clinical outcomes induced by *H. pylori* is dependent on the integrity of *cag*PAI, therefore partial deletions within *cag*PAI reduce pathogenic features (57, 58).

### *Cytotoxin-associated gene A*

According to the presence or the absence of the *cagA* gene in *H. pylori* strains two main subpopulations are considered: *cagA*-positive and *cagA*-negative strains (49). The co-existence of cytotoxin-associated gene A (CagA)-positive and negative strains in a unique host is not far from expected (59). Although CagA-positive strains cause more severe clinical outcomes which may progress to malignancy (30, 60); the results of a meta-analysis study showed that these strains are more curable (61). CagA oncoprotein is the main virulence factor of *H. pylori*. Following the adherence of *H. pylori* to the host gastric epithelial cells, the *cagA* gene is expressed (62). It was shown that CagA expression is regulated in response to environmental conditions including salt concentration, iron limitation, and pH (63-65). Via interaction with different cellular factors, CagA (128–145 kDa) interferes with numerous cellular signal transduction cascades and consequently induces gastric carcinogenesis (15, 32) (Fig. 1). Molecular anatomy shows that CagA includes a conserved N-terminal region, the variable repeats of the EPIYA (Glu-Pro-Ile-Tyr-Ala) motif, a tyrosine phosphorylation motif, and the C-terminal tail (66). According to distinct flanking amino acids around the EPIYA motifs, four different peptide segments, EPIYA-A, B, C and D have been determined (67). Almost all CagA isolates contain the EPIYA-A and B segments. EPIYA-C segment exists in CagA species that distribute in Europe, North America, and Australia; therefore, it is termed 'Western CagA'. Also, the EPIYA-D segment exists in CagA species that circulate in East Asia; therefore, it is termed 'East Asian CagA' (68). Upon delivery into the host cell, CagA may attach to the inner surface of the cell membrane and its EPIYA motifs are tyrosine phosphorylated by Src and Abl family kinases, respectively (69, 70). Subsequently, it binds Src homology 2 (SH2) domain-containing proteins (e.g., SHP2 tyrosine phosphatase and C-terminal Src kinase (Csk)) and adaptor protein Crk to disturb the adhesion, spreading, and migration of the host cell (71-74). The induction of NF-κB signalling and IL-8 secretion by CagA and CagT4SS leads to enhanced gastric inflammation, which is considered a risk factor for genetic instability and carcinogenesis (75). EPIYA-D motif's more binding tendency to SHP2 compared with the EPIYA-C motif suggests a more intense ability of East Asian CagA for induction of cellular transformations (76). Via induction of hypermethylation in the DNA promoters or histones, CagA also mediates epigenetic changes, leading to downregulation of the tumour suppressor genes (e.g., MGMT) or microRNAs (e.g., let-7) (77, 78). CagA destroys the apoptosis-stimulating protein of the p53 (ASPP2) tumour suppressor pathway and also interacts with the gastric tumour suppressor RUNX3 (RUNX family transcription factor 3), which leads to RUNX3 degradation by the proteasome (79, 80). Distinct CagA species have a different number of CagA-multimerisation (CM) motifs, also called CRPIA (conserved repeats responsible for phosphorylation-independent activity) sites, containing a 16-amino-acid sequence located in the CagA C-terminal region (81). Inside the gastric epithelial cells, the CM motif attaches to and downregulates the polarity-regulating kinase, partitioning-defective 1b (PAR1b), which is also termed the microtubule affinity-regulating kinase 2 (MARK2). The inhibition of PAR1b leads to failure of junction and polarity, which predisposes cells to oncogenesis (82). Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) are a kind of intercellular adhesion molecules that act as functional receptors for *H. pylori* outer membrane adhesion HopQ (83, 84). Recent genetic evidence showed that the interaction between HopQ and CEACAM receptors (CEACAM 1, 5, and 6) is critical for *H. pylori* CagA translocation while CagT4SS interaction with integrins (neither β1 integrin heterodimers (α1β1, α2β1 or α5β1), nor any other αβ integrin heterodimers) is not essential for this process (84-86).

### *Vacuolating cytotoxin A*

All the *H. pylori* strains have a single chromosomal copy of the *vacA* gene. This gene encodes vacuolating cytotoxin A (VacA) protein that forms intracellular vacuoles in the eukaryotic host cells (87). *vacA* gene possesses allelic polymorphism owing to the existence of the signal peptide (s1a, s1b, s1c, and s2 variants), the intermediate (i1, i2, and i3 variants), and the middle regions (m1a, m1b, m1c, and m2 variants) which cause various clinical outcomes and toxic activity in different combinations (88-92). Different combinations of s- and m-regions form four different *vacA* genotypes including s1m1, s1m2, s2m1, and s2m2; these variants have different abilities to induce vacuolisation in infected cells. The s1m1 variants are the most powerful strains in toxin

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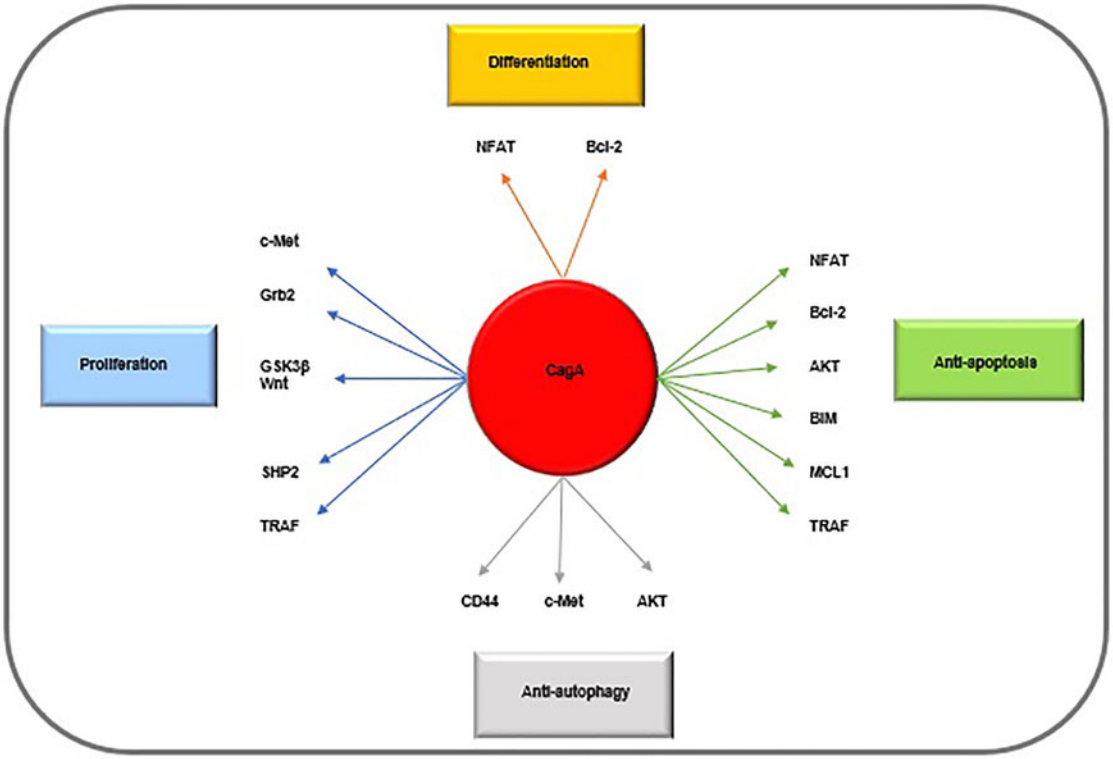


**Figure 1.** Signalling pathways induced by *H. pylori* CagA. CagA oncoprotein is directly injected into host cells by CagT4SS. This protein interacts with myriad signalling factors to manipulate different signal transduction cascades. CagA, cytotoxin-associated gene A; CagT4SS, Cag type IV secretion system; NFAT, nuclear factor of activated T cells; Bcl-2, B-cell lymphoma 2; c-Met, c-mesenchymal epithelial transition factor; Grb2, growth factor receptor-bound protein 2; GSK3β Wnt, glycogen synthase kinase 3β Wnt; SHP2, Src homology-2 domain-containing protein tyrosine phosphatase-2; TRAF, TNF receptor-associated factor; CD44, cluster of differentiation 44; Akt, Ak strain transforming; BIM, BCL-2-interacting mediator of cell death; MCL1, myeloid cell leukaemia-1.

production and vacuolisation in host cells. Cell vacuolation by s1m2 strains depends on the host cell line, s2m2 strains rarely produce cytotoxin, and s2m1 strains rarely induce cell vacuolation (91, 93-96). All s1m1i1 strains are vacuolating and all s2m2i2 are non-vacuolating. S1m2i1 strains induce cell vacuolation, whereas s1m2i2 strains could not do this. Therefore, s1m1i1 and s1m2i1 strains are more virulent and more likely associated with serious clinical outcomes such as GC compared with the s2m2i2 and s1m2i2 strains (93, 97, 98). There are extra polymorphic regions including the deletion (d)-region (d1 and d2 variants), located between the i- and the m-region, and the c-region (c1 and c2 variants) located at the 3′-end region of *vacA* gene (99, 100). The d1- and c1- genotypes are considered biomarkers of a high risk of GC (99, 100). It seems that *vacA* expression is regulated in response to host–microbe interactions, salt and iron concentrations, and low pH (101-104). The intact VacA toxin (~88 kDa) consists of an N-terminal domain (33 kDa) and a C-terminal domain (55 kDa) that are involved in cytotoxicity and binding to cell surface receptors, respectively (105, 106). This toxin consists of a signal peptide and it is secreted by the type V secretion system (T5SS, autotransporter) to the intracellular milieu (107, 108). Epithelial cell's surface receptors including (i) low-density lipoprotein receptor-related protein-1 (LRP-1), (ii) receptor-like protein tyrosine phosphatase alpha and beta (RPTP-α, -β), and (iii) sphingomyelin acts as receptors for VacA protein (109, 110). Furthermore, it binds to β2 integrin (CD18) receptors on T cells (109). VacA influences the host cells via induction of apoptosis, autophagy, membrane depolarisation, activation of mitogen-activated protein (MAP) kinases, inhibition of T-cell function and mitochondrial dysfunction contributing to *H. pylori* life-long colonisation and pathogenesis (87, 111-116). For triggering apoptosis in the gastric epithelial cells, VacA inserts into mitochondrial membranes, and subsequently, cytochrome C is released (117). VacA molecule's N-terminally encoded hydrophobic amino acids could form hexameric pores in the lysosomal, endosomal, and mitochondrial membranes of epithelial cells and phagocytes facilitating VacA's pro-apoptotic and vacuolating activity (118). Besides, VacA plays a critical role in *H. pylori* colonisation via inhibition of the proliferation and activation of B cells and T cells (119). During acute disease, VacA induces the autophagy pathways in host cells, whereas, during chronic disease, it induces the disruption of phagosomes and promotes cell vacuolation that facilitates the survival of *H. pylori* in the host epithelial cells (120, 121).

### *CagA–VacA interactions*

As *H. pylori* major virulence factors, VacA is expressed by all *H. pylori* strains, whereas CagA is exclusively expressed by specific strains (30). Deep research in bacterial life suggests a theory that bacteria may release various virulence factors able to act either in a synergistic manner or in an antagonistic manner to achieve greater compatibility with the host (122). Using a functional relationship between CagA and VacA, *H. pylori* could fine-tune its interaction with the human host stomach (122). Most of the time, VacA and CagA cellular activities are antagonistic; CagA downregulates the VacA-induced cellular vacuolation and VacA downregulates the CagA-induced cell alterations (123). One of the most important functions of VacA is the induction of autophagy which degrades the CagA protein and shortens its half-life in the host cells. VacA-induced autophagy is downregulated in gastric cells and CagA escapes degradation, such as in the cancer stem cells expressing a cell-surface marker called CD44 variant 9 (CD44v9) (124). Interestingly, a recent report provides contradictory evidence showing that in the absence of VacA, CagA undergoes both proteasomal and autophagic degradation (125). VacA causes the accumulation of dysfunctional autophagosomes

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via disrupting a late step of the autophagic pathway, which results in accumulation (rather than degradation) of CagA in the gastric epithelial cells. It seems that VacA-induced CagA accumulation in dysfunctional autophagosomes results in the vigorous limitation of CagA downstream signalling (125). CagA-induced suppression of autophagy via the c-Met-PI3 K/AKT-mTOR signalling pathway enables it to establish pro-inflammatory and carcinogenic action (126). CagA obstructs the internalization of VacA to the host cells and leads to the blocking of VacA-induced apoptosis (127). CagA activates calcineurin and subsequently activates the transcription factor nuclear factor of activated T cells (NFAT), which exerts pleiotropic actions on cell proliferation and differentiation. Whereas VacA downregulates the NFAT by decreasing the calcium influx blocking calcineurin activation (128). However, the co-participation of VacA and CagA in the iron acquisition by *H. pylori* is a rare example of their synergistic effect (129).

### Urease

*H. pylori* urease is involved in the survival and colonisation of the bacterium and induces infection in the stomach (130, 131). A gene cluster containing seven genes is regulated by two promoters that encode the *H. pylori* urease enzyme. The *ureA* and *ureB* genes (encoding catalytic units) are regulated by the first promoter. Downstream genes, including *ureI* (encoding acid-gated urea channel) and *ure E–H* (encoding accessory assembly proteins), are under the control of the second promoter (132, 133). In *H. pylori* species, urease activity is regulated by the availability of the cofactor nickel and the pH of the stomach microenvironment (134, 135). It was shown that the existence of nickel in the bacterial culture medium dramatically increases urease activity (135). Besides, the acid-gated urea channels are closed at pH 7.0 and are firmly open at pH 5.0 (31, 134). Urease is a vital virulence factor to the survival of *H. pylori* in the human host stomach; recently, Madison *et al.* demonstrated that in response to nitric oxide (NO), as a product of the host innate immunity, CrdRS two-component system (TCS) regulates the expression of *ureA* gene (136). Urease is a polymeric enzyme (5–10% of the total protein content) that is composed of two subunits, i.e., UreA (29.5 kDa) and UreB (66 kDa), of which UreB is considered the subunit responsible for the enzyme activity (137). It was shown that a cluster of the 12 active sites containing 24 nickel ions on the urease supramolecular structure guarantees enzymatic action and *H. pylori* survival at low pH (138). The urease enzyme catalyses the urea conversion to ammonia and carbon dioxide, raising the acidic stomach pH to neutral to protect *H. pylori* from acidity via the formation of a cloud of ammonia which neutralises acidic pH (139). Following the production of ammonia, a soluble form of occludin, a 65-kDa tetraspan integral membrane protein, is accumulated which leads to the disruption of tight epithelial junctions (140). Besides, high levels of ammonia cause cytotoxic effects on the gastric epithelial barrier and ruin these cells' mitochondrial oxygenation (141). Natural pH generated by the urease enzyme reduces mucin viscoelasticity to convert the gastric mucin structure from gel to sol, leading to bacterial free movement through the mucus (142). Based on localisation, there are internal and external types of urease enzymes. The internal urease is produced by live bacterial cells and is active at pH 2.5–6.5, whereas the external urease is produced during cell lysis and is active at pH 5.0–8.5 (143). Increased urease activity may lead to a higher risk of induction of histopathological alterations within the gastric mucosa and greater gastric carcinogenesis (144).

### Helicobacter outer membrane porins

*H. pylori* adherence to the gastric epithelium is necessary for the delivery of toxins (e.g., CagA and VacA) or other virulence factors into the host cells, which results in inflammatory or immune response-mediated direct or indirect damages (60). The interaction between the receptors expressed on the gastric epithelial cells and Helicobacter outer membrane porins (Hop) family, adhesion factors encoded by *hop* genes, starts *H. pylori* infection (145). Hop family porins including HopS, HopP, HopH, HopQ, and HopZ augment *H. pylori* adherence to the host cell and boost inflammation by promoting the expression of virulence factors and the secretion of inflammatory cytokines (146). Widely studied among the Hop family are blood group antigen-binding adhesin (BabA) and sialic acid-binding adhesin (SabA) (147). A wide variety of receptors for BabA and SabA binding activity have been found in the human host stomach and saliva (146, 147). BabA (HopS) binds to H-type 1 and ABO/Lewis$^{b}$ (Le$^{b}$) blood group antigens exposed on the gastric epithelium and mucus layer (148). According to X-ray analysis, it was shown that BabA contains three structural domains including one conserved loop (CL2) and two diversity loops (DL1 and DL2) for interaction with Le$^{b}$ (149). BabA protein has two domains including the extracellular N-terminal domain linked to Le$^{b}$ antigens and an outer membrane C-terminal domain that anchors into the outer membrane (150). BabA not only contributes to bacterial adherence and colonisation but also augments a nonspecific immune including granulocyte infiltration or secretion of IL-8 enhancing gastric inflammation (151). BabA adherence to fucosylated Le$^{b}$ antigen facilitates CagT4SS activity which leads to the release of high amounts of the pro-inflammatory factors inducing carcinogenesis (148, 152). Low BabA production and decreased binding tendency to the Le$^{b}$ leads to detachment of *H. pylori* from the gastric mucus and induces ulceration within the duodenum and consequently increases the risk of PUD (153). The existence of BabA in Western countries is correlated to the high prevalence of PUD and GC, but there is no such correlation in Asians (154). SabA (HopP) is an adhesion molecule, which is involved in *H. pylori* binding and colonisation via the interaction with sialyl-Le$^{x}$, sialyl-Le$^{a}$ , and Le$^{x}$, but not with other Lewis's antigens, such as Le$^{a}$, Le$^{b}$ or Le$^{y}$ (155, 156). SabA-expressing strains could promote gastric diseases, redundant neutrophil infiltration and gastric atrophy during infection; furthermore, these strains could vastly colonise (157, 158). Recently, it was shown that the tropism of BabA along with SabA for spasmolytic polypeptide-expressing metaplasia (SPEM) glands, enables *H. pylori* to induce metaplastic alterations and trigger the onset of carcinogenesis (159). *H. pylori*'s successful colonisation and persistent infection are largely dependent on two major adhesins: including BabA and SabA. Therefore, these adhesins can be considered potential vaccine candidates against *H. pylori* (147, 160-162).

### γ-Glutamyl-transpeptidase

γ-Glutamyl-transpeptidase (GGT) is a 61 kDa protein that catalyses the conversion of glutamine into glutamate and ammonia as well as glutathione into glutamate and cysteinyl glycine. This enzyme enables *H. pylori* to take up and incorporate glutamate into the tricarboxylic acid (TCA) cycle (163). Initially, it was thought that GGT involves in *H. pylori* colonisation but more studies confirmed that GGT knockout mutant strains can colonise animals (164, 165). The GGT expression and activity may promote the development of peptic ulcer disease (PUD) (166). The apoptotic effect on the host cells has been recognised for *H. pylori* and *H. suis* GGT (167). However, it seems that GGT is not the main factor in the induction of *H. pylori*-mediated apoptosis in T cells because a GGT-negative mutant still induces T-cell apoptosis (168). *H. pylori* GGT induces cell cycle arrest at the G1/S phase transition in T cells and gastric epithelial cells, and subsequently inhibits their proliferation (169, 170). It was demonstrated that GGT and VacA could inhibit T cells which emphasises the role

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of secreted virulence factors in the *H. pylori* pathogenesis (168). GGT and VacA promote immune tolerance and gastric persistence that facilitate the prevention of asthma *in vivo* (165, 171).

### *Tumour necrosis factor-alpha (TNF-α)-inducing protein*

Various *H. pylori* strains have a conserved sequence of *tipα* gene encoding tumour necrosis factor-alpha (TNF-α)-inducing protein (Tipα) as a small and secretory protein (172). It was shown that Tipα has a weak homology to Gram-positive bacterial penicillin-binding proteins. This led us to conceive that the *tipα* gene has been derived from Gram-positive bacteria and transferred horizontally to *H. pylori* (173, 174). The Crystal Structure of the Tipα indicates that the functional Tipα protein (approximately 37 kDa) is a homodimer one (175, 176). According to the well-established data, a functional T4SS induces epithelial cells to produce cytokines against *H. pylori* infection (177). In addition to the mentioned pathway, it was found that Tipα is a strong inducer of pro-inflammatory cytokine and chemokine gene expressions (178). Through the activation of nuclear factor kappa B (NF-κB), Tipα induces the overexpression of TNF-α in the Bhas 42 (BALB/3T3 cells transfected with v- H-ras gene) and MGT-40 cells (mouse gastric epithelial cell line) (174, 179). TNF-α is well characterised as a master tumour promotor. It is a key regulator of inflammation that plays a critical role in the cytokine network between cancer and inflammation (180). Accordingly, Tipα is a potent carcinogenic factor that could induce inflammation and/or CG, hyperplasia, and GC in individuals infected with *cag*PAI-negative *H. pylori* strains (174, 179, 181). Tipα plays a critical role in the colonisation of mouse gastric mucosa (182), however, its secretion is independent of the CagT4SS (179). Tipα can bind a membrane receptor called nucleolin and subsequently, it can internalise into the gastric epithelial cell's cytoplasm (183). Interestingly, the external ligands of nucleolin including hepatocyte growth factor (HGF), K-ras and Tipα are carcinogenic (184). Tipα interacts with single- and double-strand forms of DNA in the GC cells suggesting that DNA binding may have a possible role in the molecular mechanisms of carcinogenesis (185). Turning to a monomer form leads to the loss of Tipα's functional performances in tumour promotion, TNF-α induction and NF-kB activation (174, 179). Besides, the monomer form of Tipα has poor DNA-binding ability (186).

### *Morphological transformation*

*H. pylori* is typically known as spirally twisted rods, whereas its highly heterogenic nature can form various cell shapes, including coccoid forms, elongated (filamentous) forms, and straight or curved rods (187). During the morphological transition to coccoid forms, *H. pylori* loses its culturability which could be a sign of bacterial death. Using highly advanced genetic and microbiological techniques, it was shown that these cells are alive and they have modified their physiology (188). Decreased cell size and dramatic limitations in metabolic activity lead to *H. pylori* morphological transformation into coccoid form, which translates into a transition to a viable but non-culturable (VNC) phenotype (188). Spiral viable culturable form (SVCF) and coccoid viable but non-culturable form (CVNCF) are two distinct forms distinguished via molecular techniques and electron microscopy (189). However, several studies have proposed that coccoid forms colonise the mucus layers, produce virulence factors, escape the immune responses, promote carcinogenesis, and play a critical role in therapeutic failures (33, 190-195). Therefore, blocking the process of morphological transformation of *H. pylori* might be a promising strategy for the successful eradication of this pathogen (187, 193). Myricetin (MYR; 3,5,7,3′,4′,5′ hexahydroxyflavone, a natural anti-virulence compound) interferes with the morphological transformation of *H. pylori* from spiral/rod-shaped forms to coccoid forms and increases the activity of antibiotics against this pathogen (196). It was shown that following exposure to MYR, genes related to the *H. pylori* morphogenesis (e.g., *csd3*, *sd6*, *csd4*, and *amiA*) were downregulated. These suppressed genes are mostly involved in the shortening of muropeptide monomers, suggesting their major role in the spiral-to-coccoid transition (196). Cultivation under mild sub-optimal growth conditions such as acidic and alkaline pH, high temperature, aerobiosis, extended incubation, and exposure with a proton pump inhibitor and/or antibiotics stimulates *H. pylori* morphological conversion from spiral to coccoid (188, 197-202). It is postulated that commensal bacteria undergo an increased cell filamentation, whereas pathogenic bacteria undergo a reduction of elongation (203). In agreement with this hypothesis, it was demonstrated that highly virulent strains had shorter cells than the lower virulent strains; indicating that adaptational changes in cell morphology drastically associate with the virulence profile of *H. pylori* strains (204).

## Interaction between *H. pylori* and the human host immune system: a life-long challenge

Although *H. pylori* was originally considered an extracellular germ, it was shown that a small portion of this pathogenic bacteria can penetrate the intracellular compartments of various cell types (205, 206). It seems that the intracellular lifestyle protects *H. pylori* from the antibiotics and host immune responses which facilitate persistent infection (207). Despite the induction of intense pro-inflammatory response by various Gram-negative bacteria, *H. pylori* stimulates only a relatively feeble immune response which results in asymptomatic and persistent infection. Various unique immune evasion strategies mediated by numerous virulence factors are raised *H. pylori*-derived life-long infection (208, 209). These strategies include (i) survival in a harsh habitat, (ii) changing macrophages and DCs functions, (iii) induction of the secretion of the anti-inflammatory cytokines, and (iv) the stimulation of Treg cell development (210-213). It was shown that after exposure to live *H. pylori*, human monocytes initially secret a mixture of pro- and anti-inflammatory cytokines, whereas the prolonged-term innate memory promotes the secretion of anti-inflammatory cytokines (212).

### *Innate immunity evasion strategies*

#### *Escape from innate immune recognition*

The highly conserved pathogen-associated molecular patterns (PAMPs) expressed by *H. pylori* are recognised by PRRs that are expressed by the innate immune cells and gastric epithelial cells (214). The interactions between PAMPs and PRRs trigger innate immune responses which are followed by adaptive immune responses (215, 216). PRR families are classified into transmembrane receptors (Toll-like receptors (TLRs) and C-type lectin receptors (CLRs)) and intracellular receptors (nucleotide-binding oligomerisation domain- (NOD-) like receptors (NLRs) and retinoic acid-inducible gene- (RIG-) I-like receptors (RLRs)) (217, 218). PAMPs sensation by PRRs activates intracellular signal transduction pathways triggering stormy reactions such as antimicrobial activity and inflammatory response to eliminate the pathogenic microorganism (217). The TLRs are major classes of PRRs involved in the recognition of PAMPs, such as lipopolysaccharide (LPS), lipoteichoic acid (LTA), hypo-methylated CpG-rich regions of DNA, lipoprotein (LP), flagellin and PG (219). Numerous scientific reports explain the role of TLRs during *H. pylori* infection (220-222). Since several PAMPs of *H.*

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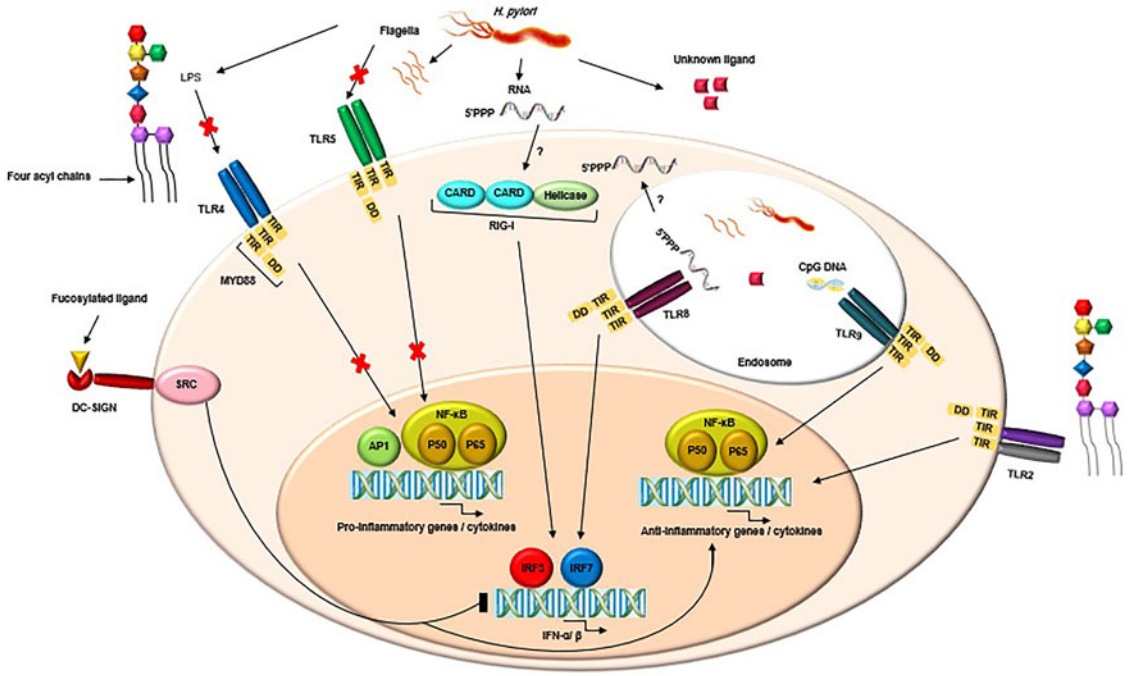


**Figure 2.** *H. pylori* evasion of innate immune recognition. Structurally modified pathogen-associated molecular patterns (PAMPs) enable *H. pylori* to evade the detection by pro-inflammatory Toll-like receptors (TLRs). *H. pylori* tetra-acylated LPS is less biologically active and is not sensed by TLR4. The TLR5 cannot detect the mutated TLR5 binding site of *H. pylori* flagellin. TLRs 9 detects *H. pylori* DNA (CpG DNA) and TLR2 detects *H. pylori* LPS; these TLRs predominantly activate anti-inflammatory signalling pathways and IL-10 expression. The cytosolic receptor RIG-I and endosomal receptor TLR8 sense 5′ triphosphorylated RNA which elicit IFN-α/β response. *H. pylori* fucosylated DC-SIGN ligands are another activator of anti-inflammatory genes. Besides, theses ligands block IFN-α/β response. Notice that the different cell types express various pattern recognition receptors (PRRs). TLR2/4/5/8/9, Toll-like receptor 2/4/5/8/9; LPS, lipopolysaccharide; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin; SRC, steroid receptor coactivator; IFN-α/β, α/β interferon; IRF3/7, interferon regulatory factor3/7; AP-1, activator protein 1; NF-κB, nuclear factor-κB; P50/P65, NF-κB p50/p65 heterodimer; TIR, Toll/interleukin-1 receptor domain; MYD88, myeloid differentiation primary response gene 88; DD, death domain; CARD, caspase activation and recruitment domain; RIG-I, retinoic acid-inducible gene I, CpG DNA, 5'—C—phosphate—G—3'.

*pylori* trigger anti- but not pro-inflammatory responses, *H. pylori* could evade innate and adaptive immune responses to survive and persist in life-long infection (Fig. 2) (223). *Escherichia coli* hexa-acylated LPS with a negative charge and potent immunostimulatory properties activates inflammatory signalling via the sensation of TLR4, whereas *H. pylori* tetra-acylated LPS with less negative charge could not be recognised by TLR4 and resists antimicrobial peptides (224). Recently, Schmidinger *et al.* found that lipid A part of *H. pylori* LPS interacts with human annexins and dramatically suppresses LPS-mediated TLR4 signal transduction which subsequently hinders the innate immune system (225). The N-terminal position of flagellin in *Salmonella enterica* could be detected by TLR5, but this critical position (TLR5 binding site) has mutated in *H. pylori* (226). Additional *H. pylori* PAMPs-PRRs interactions include the detection of *H. pylori* hypo-methylated CpG DNA by endosomal TLR9, and sensation of *H. pylori* LPS by TLR2 (217, 227, 228). These two TLRs induce anti-inflammatory and tolerogenic responses causing persistent infection (229-231). *H. pylori* 5′ triphosphorylated RNA is recognised by endosomal TLR8; this triggers a downstream signalling pathway that results in the transactivation of type I interferon (IFN-I (IFN-α/β)) in human monocytes (217, 232). *H. pylori* 5′ triphosphorylated RNA is also detected by one kind of RLRs known as RIG-I. The stimulation of the RIG-I receptor leads to the activation of the transcription factors IRF3 and IRF7 and subsequent expression of IFN-I (233). According to *in vitro* and *ex vivo* experiments, recently it was shown that through the downregulation of IRF3 activation, *H. pylori* actively inhibits cyclic GMP-AMP (cGAMP) synthase (cGAS)-stimulator of interferon genes (STING) and RIG-I signalling (234). DCs-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) is a member of the CLR family which plays a critical role in *H. pylori* recognition and pathogenesis. DC-SIGN ligands are mannosylated in most of the pathogens causing activation of the pro-inflammatory pathways. Although DC-SIGN ligands of *H. pylori* are fucosylated leading to the suppression of the signalling pathways downstream of DC-SIGN and also causing activation of the anti-inflammatory genes (235). The macrophage-inducible C-type lectin (MINCLE) is another CLR, it interacts with the Lewis antigens of *H. pylori* LPS inducing macrophages to secrete low levels of TNF-α and high levels of IL-10 promoting *H. pylori* persistence (236). NOD1 (CARD4) and NOD2 (CARD15) are two members of NLRs that can recognise various motifs in PG of various Gram-negative pathogenic bacteria (237, 238). PG delivered by the *H. pylori* CagT4SS is sensed by NOD1 in the cytoplasm of epithelial cells and causes the activation of NF-κB signalling and upregulation of pro-inflammatory immune responses (55). It was shown that the immunomodulatory glycoprotein olfactomedin 4 (OLFM4) targets NOD1 and NOD2 in *H. pylori*-infected cells (239). OLFM4 is a target gene of the NF-κB pathway and associates directly with both NOD1 and NOD2 proteins thereby having a negative feedback effect on NF-κB activation induced by *H. pylori* infection. It was shown that the knockout of the OLFM4 gene in mice reduces *H. pylori* loads and increases gastric immune cell infiltration. Via a negative regulatory effect on *H. pylori*-specific NOD-mediated immune responses, OLFM4 plays an impressive role in the persistence of *H. pylori* colonisation (239). The bacterial ligand such as PG can access intracellular NOD-1 by CagT4SS and outer membrane vesicles (OMVs) (240). PG deacetylation allows *H. pylori* to evade host innate immunity via induction of an NOD-1-dependent negative feedback loop. It seems that NOD-1 alters macrophage

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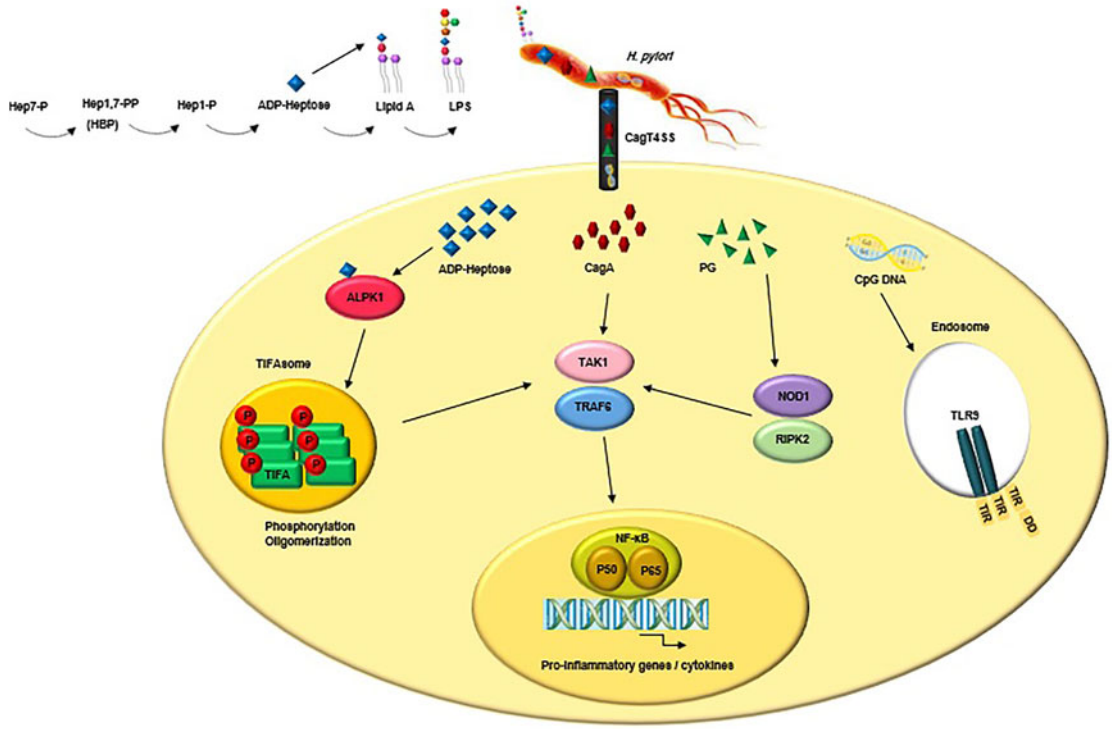


**Figure 3.** CagT4SS-injected virulence factors (e.g., ADP-Heptose, CagA and PG) trigger NF-κB-mediated innate immune response in gastric epithelial cells. Upon binding of ADP-Heptose, an intermediate metabolite produced during the biosynthesis of LPS, to ALPK1, the ALPK1-TIFA signalling pathway is triggered, which results in an NF-kB-dependent pro-inflammatory response. Following TIFA activation, PG activates NOD1, which leads to NF-κB-mediated pro-inflammatory responses. Finally, the interaction of CagA and host TAK1 triggers NF-κB-mediated pro-inflammatory responses. Besides, CagT4SS-injected CpG DNA is detected by TLR9 which result in activation of anti-inflammatory signalling pathways. LPS, lipopolysaccharide; CagT4SS, Cag type IV secretion system; ADP-Heptose, ADP-β-D-*manno*-heptose; PG, peptidoglycan; CagA, cytotoxin-associated gene A; ALPK1, alpha kinase 1; Tak1, TGF-β activated kinase 1; TRAF6, tumour necrosis factor receptor (TNFR)-associated factor 6; TIFA, TRAF interacting forkhead-associated protein A; NOD1, nucleotide-binding oligomerisation domain 1; RIPK2, receptor-interacting-serine/threonine-protein kinase 2; NF-κB, nuclear factor-κB; P50/P65, NF-κB p50/p65 heterodimer; CpG DNA, 5'—C—phosphate—G—3'; TLR9, Toll-like receptor 9; TIR, Toll/interleukin-1 receptor domain; DD, death domain.

polarisation leading to *H. pylori* persistence (241). Bacterial sugars including ADP-β-D-manno-heptose (ADP-Heptose) and D-glycero-β-D-manno-heptose-1,7-bisphosphate (βHBP) are key intermediate metabolites of LPS inner heptose core. The biosynthesis of ADP-Heptose and βHBP is a novel potent and *cag*PAI-mediated cell activation pathway triggering NF-κB augmentation and IL-8 release in human epithelial cells (242, 243). Upon CagT4SS-mediated internalization of βHBP into the gastric epithelial cells, it is sensed by α-kinase 1 (ALPK1) and TNF receptor-associated factors (TRAF)-interacting protein with forkhead-associated domain (TIFA). The activation of the ALPK1-TIFA signalling axis leads to the activation of NF-kB signalling (242-244). Recently, Maubach *et al.* showed that the TIFA has dual functions in *H. pylori*-induced NF-κB pathways; induction of NF-κB classical pathway via the association of TIFA with TRAF6, and induction of NF-κB alternative pathway via the association of TIFA with TRAF2/TRAF3 (245). The TIFA is an intrinsic anti-parallel dimer, containing a threonine residue (Thr9), a central forkhead domain (FHA), and a C-terminal TRAF6 binding site (T6BP). Following βHBP sensation, Thr9 is phosphorylated and then intermolecular pThr9–FHA interactions lead to head-to-tail oligomerisation. The TIFAsome (including TIFA oligomers, TRAF2, and additional host factors) recruits and activates TRAF6, triggering NF-κB activation and subsequent inflammatory signalling (246). Following TIFA activation, PG delivered through the CagT4SS activates NOD1 which leads to NF-κB-mediated pro-inflammatory responses. Within hours of infection and before NOD1 activation, the ALPK1-TIFA signalling pathway is activated and triggers strong NF-κB-dependent inflammation. Finally, the interaction of CagA (delivered through the CagT4SS) and host transforming growth factor (TGF)-β-activated kinase 1 (TAK1) triggers NF-κB-mediated pro-inflammatory responses (247). Fig. 3 illustrates the model of *H. pylori* CagT4SS-mediated, NF-κB-driven innate immune response in gastric epithelial cells. The successive activation of the ALPK1-TIFA signalling pathway and NOD1, and CagA delivery trigger the initial inflammatory response in gastric epithelial cells, motivate the subsequent recruitment of immune cells and lead to CG (247); however, the contribution rates of these pathways to natural infection are not clear. It was reported that mutation in the genes required for the synthesis of βHBP (e.g., *rfaE*, gene HP0858 in strain 26 695) dramatically reduces IL-8 induction (>95%) and ruins CagT4SS-dependent cellular signalling (43, 248). It was shown that ADP-Heptose, a derivative of βHBP, was present in *H. pylori* at more concentration (10 times) than its origin. Simultaneously, ADP-Heptose was dramatically more potent and cells distinctly recognise the existence of the β-form. The aforementioned findings revealed that ADP-Heptose is not only a new potent NF-kB-activating PAMP in *H. pylori* but also it is a general Gram-negative bacteria-derived PAMP (242). Furthermore, ALPK1 is a cytosolic innate immune receptor for bacterial ADP-Heptose (244). The activation of the ALPK1-TIFA-NF-kB axis and CagA translocation are two distinct functions of CagT4SS because, despite the mutation in bacterial *rfaE* or the host ALPK1, CagA internalization continues (243). It was demonstrated that *cag*PAI proteins enhance the colonisation rate via the suppression of antimicrobial peptides (249). The inverse correlation between gastric β-defensin 1 level and colonisation in *H. pylori* carriers is attributed to the reduced expression of human β-defensin 1 in a CagT4SS-dependent manner

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(249). β-defensin 3 is another human antimicrobial peptide, with strong anti-*H. pylori* activity. At the beginning of *in vitro* infection, β-defensin 3 is induced in an MAP kinase- and epidermal growth factor receptor (EGFR)-dependent manner. The induction of β-defensin 3 is followed by the stable shutdown through CagA-mediated activation of the Src homology domain, containing protein tyrosine phosphatase 2 (SHP2) and downmodulation of the EGFR signalling pathway (250).

### Manipulation of innate immune cells

The innate immune cells in the lamina propria and the mucus layer on the surface of gastric epithelial cells are the main defensive barrier against *H. pylori* (251). *H. pylori* CagA in macrophages interacts with SHP-1 and targets TRAF6 for K63-linked ubiquitination, thereby this multi-functional protein can downregulate the expression of pro-inflammatory cytokines and subsequent immune response (252). Gang Liu *et al.* showed that *cagA*-positive *H. pylori* strains abrogate Cathepsin C (CtsC) to ruin neutrophil activation; this process prevents bacterial clearance and guarantees persistent infection (253). It was shown that the phosphorylated form of CagA over-expresses the gene encoding haem oxygenase-1 which leads to macrophage anti-inflammatory responses and causes persistent infection (254). It was demonstrated that CEACAM1 expressed mainly by activated immune cells, including NK cells and T cells acts as a suppressive receptor (255). Recently, Gur *et al.* showed that the secretion of IFN-γ by CD4+ T cells is suppressed by HopQ; also, NK cell and T-cell functions are inhibited by HopQ-mediated activation of CEACAM1 which may result in immune cells' harness (256). HopQ–CEACAM interaction modulates the expression and secretion of chemokines in immune cells which leads to *H. pylori*'s survival within neutrophils in a HopQ-dependent manner (85). Via the suppressive effects on DCs and inductive effect on macrophages, VacA suppresses IL-23 expression and induces IL-10 and TGF-β secretion. The VacA immunomodulatory activity built a tolerogenic environment for *H. pylori* and leads to a chronic infection (213). The reduction of proliferation of immune cells, including neutrophils, macrophages, eosinophils, DCs, B cells, and T cells is also attributed to the VacA protein (87, 257, 258). VacA downregulates class II major histocompatibility complex (MHC II)-dependent pathways and via the formation of vesicular compartments inside macrophages assists *H. pylori*'s intracellular survival (259). NapA manipulates both innate and adaptive immune responses by: (i) upregulation of MHC II, (ii) promotion of T helper1 (Th1) cell differentiation, and (iii) stimulation of IL-12 and IL-23 release from neutrophils and monocytes (260). Another potent immunomodulator is the urease enzyme; it (i) modifies opsonisation, (ii) augments the chemotaxis of neutrophils and monocytes, (iii) binds to the MHC II receptors, and subsequently facilitates apoptosis, and (iv) increases the secretion of the pro-inflammatory cytokines (261). GGT downregulates T-cell proliferation and DCs differentiation (165, 169, 262, 263). To achieve life-long survival in the host, *H. pylori* downregulates macrophage phagocytosis and inhibit NO production by the induction of macrophage arginase II (ARG2) (264, 265). Inhibition of cell division-associated genes by *H. pylori* attenuates the proliferation of macrophages (266). Besides, *H. pylori* promotes mitochondrial membrane depolarisation and hydrogen peroxide secretion which induce apoptotic program in macrophages (267, 268). Persisting a life-long *H. pylori* infection in the host depends on the inhibition of macrophage-mediated functions including phagocytosis, human leukocyte antigen-II (HLA-II) expression and IFN-γ production which results in T-cell suppression (269). Via the glucosylation of cholesterol, *H. pylori* escapes from macrophage phagocytosis and could survive (270). Morey *et al.* showed that *H. pylori* depletes cholesterol in gastric glands to inhibit IFN-γ signal transduction and evade inflammatory response (271). Antigen presentation to T cells is one of the most important functions of DCs; abrogation of this function by *H. pylori* blocks Th1 cell differentiation (272).

### Adaptive immunity evasion strategies

Despite the high prevalence of *H. pylori* infection among the human race, the majority of the infected individuals are asymptomatic for life, and only a minority of them develop *H. pylori* infection-related disease (223, 273). Studies on human carriers and mouse models show that the polarisation and severity of the *H. pylori*-specific Th cell responses are major predictors and drivers of the disease (274). Compared with the asymptomatic carriers, the PUD patients have a threefold higher anti-*H. pylori* Th1 cell response, twofold lower Treg response, sixfold higher Th2 response, and dramatically reduced levels of TGF-β and IL-10 in the gastric mucosa (275). This imbalance suggests an association between inadequate Treg response and the development of *H. pylori*-derived disease (275). It was shown that the severity of gastritis has an inverse correlation with the number of gastric Tregs and the cytokines secretion by Tregs (276). These findings are in line with previous work showing that Tregs gather in the gastric mucosa and quench exclusive memory T-cell responses in the infected but not uninfected patients (277, 278). Treg responses to the infection are predominantly launched in the asymptomatic (healthy) carriers resulting in effectively damping immunopathologic reactions and promotion of the persistent infection. Although T-effector-dominated responses are predominantly expressed in symptomatic carriers that promote the disease (223). Using two critical cytokines including IL-10 and TGF-β, Treg cells can inhibit T-effector cell-driven immunopathology (274). Thus, the expression level of these cytokines in the gastric mucosa could effectively predict *H. pylori*-induced clinical outcomes (223). It was shown that the downregulation or neutralisation of IL-10 signalling could effectively trigger strong T-cell-dependent immunopathology and clear *H. pylori* (279). Comparison of T-cell responses of the symptomatic versus asymptomatic carriers as well as children with mild gastritis versus adults with severe gastritis suggests that Treg/T-effector cell ratios are associated with the clinical outcome (275, 276). Since vaccination is the only effective strategy to achieve protective immunity, understanding the importance of T-effector versus Treg responses to establish *H. pylori* clearance or immunopathology is a major issue in *H. pylori* vaccinology (280). For successful colonisation, *H. pylori* must suppress effector T cells (Th1 and Th17 subsets) activity, proliferation and clonal expansion. GGT and VacA are two critical virulence factors devastating T-cell-mediated immunity (223) (Fig. 4). β2 integrin subunit of the heterodimeric transmembrane receptor lymphocyte function-associated antigen-1(LFA-1) acts as a receptor for hexameric VacA (281). Subsequent ligand–receptor binding, VacA is entered upon protein kinase C-mediated serine/threonine phosphorylation of the β2 integrin cytoplasmic tail (282). Cytoplasmic VacA blocks NFAT dephosphorylation by the $Ca^{2+}$/calmodulin-dependent phosphatase calcineurin. Then the nuclear transfer of NFAT was prevented resulting in the downregulation of IL-2 production as well as subsequent T-cell activation and proliferation (223). GGT could arrest T-cell proliferation in the G1 phase of the cell cycle through the disruption of the Ras signalling pathway (207). Using a cAMP-dependent pathway, VacA and GGT trigger the secretion of miR-155 and Foxp3 in the human lymphocytes (283). The GGT enzymatic activity is linked to its immunomodulatory effects, whereas VacA vacuolating cytotoxicity is independent of its immunomodulatory effects, as both the non-toxigenic (s2/m2) or toxigenic (s1/m1) types of VacA are equally

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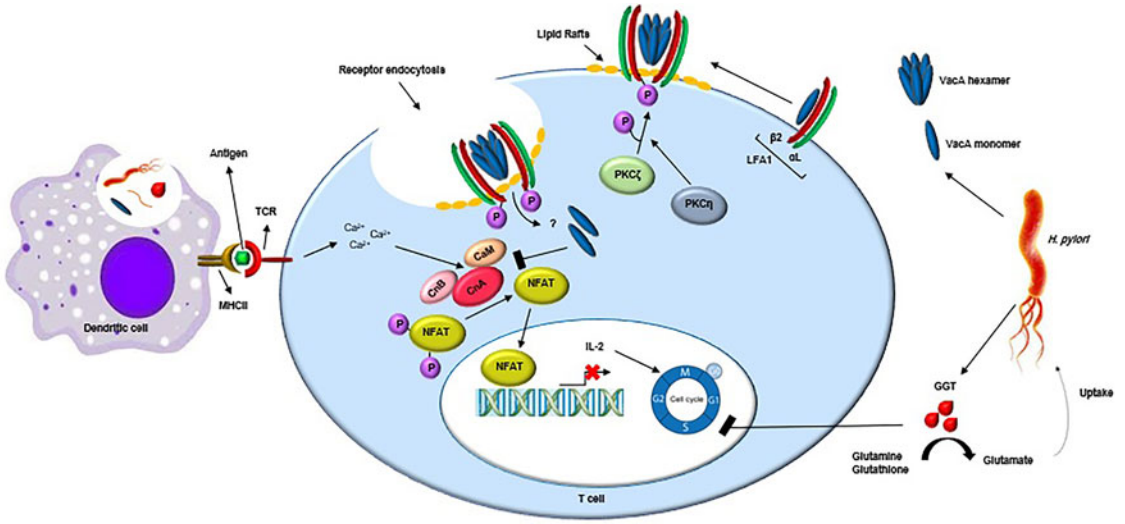


**Figure 4.** *H. pylori* subverts T-cell-mediated immunity using the secreted virulence factors VacA and GGT. Following the internalization, Cytoplasmic VacA prevents nuclear translocation of NFAT by inhibiting its dephosphorylation by the $Ca^{2+}$/calmodulin-dependent phosphatase calcineurin and thereby blocks IL-2 production and subsequent T-cell activation and proliferation. The GGT prevents the proliferation of T cells via interfering in the G1 phase of the cell cycle. TCR, T-cell receptor; MHCII, major histocompatibility complex class II; VacA, vacuolating cytotoxin A; GGT, γ-glutamyl transpeptidase; NFAT, nuclear factor of activated T cells; IL-2, Interleukin-2; CnA/B, calcineurin A/B subunits; CaM, calmodulin; LFA-1, lymphocyte function-associated antigen-1; PKCζ/ η, protein kinase Cζ/η.

tolerogenic *in vitro* (165). Selective recruitment or activation of Tregs in the preferred niche facilitates the promotion of chronicity by persistent pathogens, such as *Mycobacterium tuberculosis* and certain helminths (284). The same is true for *H. pylori*, which establishes Treg-mediated immunosuppression promoting chronic infection (274, 275). *In vitro* and *in vivo* findings imply that *H. pylori* not only triggers DCs-derived tolerogenic (i.e., Treg-inducing) responses but also quenches their immunogenic functions (223). Mature and immature DCs diversely affect the Th cells differentiation which results in immunity or immunosuppression (Fig. 5). DCs maturation is promoted via the cytosolic or membrane-bound PRRs-mediated recognition of PAMPs. Mature DCs can express high levels of MHC II, maturation markers, co-stimulatory markers (e.g., CD80, CD86, and CD40), Th cell-activating cytokines, and Th cell-differentiating cytokines as well as other pro-inflammatory cytokines (e.g., IL-12, IL-23, TNF-α, and IL-6) (210). Differentiation of naive T cells into Th1 or Th17 cells depends on antigen recognition via the T-cell receptor, soluble cytokine signals, co-stimulatory signals, and high-level of IL-12 and IL-23 (223). This scenario will result in immunity and *H. pylori* control. *H. pylori*-exposed DCs remain immature and present tolerogenic activity. Despite the high expression of MHC II by the semi-mature DCs, these cells fail to express co-stimulatory markers and Th1/Th17 cells differentiating cytokines, and instead, they produce high levels of the anti-inflammatory cytokine IL-10 (285). These semi-mature DCs efficiently induce Treg differentiation and immunosuppression *in vitro* and *in vivo* (285); however, LPS treatment can break the tolerance *in vivo* (285). The *H. pylori* virulence and persistence factors including VacA and GGT act as the inhibitors of murine DCs maturation and tolerogenic reprogramming (285); CagT4SS is their counterpart, which acts on human DCs (210). Mutation of either GGT or VacA (i) reduces the colonisation of mutant strains in mice, (ii) suppresses the prevention of LPS-induced DCs maturation, and (iii) inhibits the DCs tolerisation (165). Via a partial or total inhibition of activation of T cells in the lamina propria, VacA proteins can subvert the immune response (33, 121). All *in vivo* findings suggest that under *H. pylori* forces, DCs present tolerogenic properties leading to the anti-inflammatory cytokine secretion and Treg differentiation, as well as downregulation of T-effector cell function, and establishment of persistent infection (223). Tregs facilitate the persistence of infection and protect the infected host cells against gastric inflammation. Besides, Tregs encourage bacterial colonisation which likely promotes gastric tumour progression (286).

## Vaccine development against *H. pylori*

Owing to high antibiotic resistance among *H. pylori* strains, World Health Organization (WHO) put this bacterium on its high-priority pathogen list to prioritise research and development on the deadly threats to global health (287). Owing to increasing antibiotic resistance, high cost, poor patient compliance, and re-infection the current therapeutic strategies remain sub-optimal (288). Besides, GC is the most drastic clinical outcome of *H. pylori* infection, and it is ranking fifth for incidence and fourth for mortality globally (289). Therefore, priority for *H. pylori* eradication comes with vaccination (1, 41, 290). Until now various kinds of vaccines including (i) inactivated whole-cell vaccines, (ii) subunit and recombinant protein vaccines, (iii) epitope-based vaccines, (iv) DNA vaccines, and (v) vector vaccines have been introduced for vaccination against *H. pylori* (291-295) (Fig. 6). Reviewing the recent progress on *H. pylori* vaccines shows that these efforts have not been very fruitful and most vaccine candidates are at a very early stage (phase I or even preclinical) (41, 296, 297). *H. pylori* evasion from the host adaptive immunity and its persistent infection are attributed to extreme adaptation to the gastric environment and result in current vaccines' partial or limited protectivity (3, 298). There is no agreement on the role of humoral immunity in *H. pylori* vaccine-induced protective immunity (299, 300). Nevertheless, Sun *et al.* showed that Th1 and Th17 cell responses induced by immunodominant antigens protect mice against *H. pylori* infection which confirms the vital role of cellular immunity for the clearance of *H. pylori* infection (301). Acquisition of *H. pylori* infection occurs in early childhood, so an effective vaccine should protect individuals for 10–15 years and more (302). *H. pylori's* successful colonisation, persistence and induction of severe clinical outcomes in the human host is dependent on passing through several steps (31). Designing and developing multivalent and multistage vaccines containing

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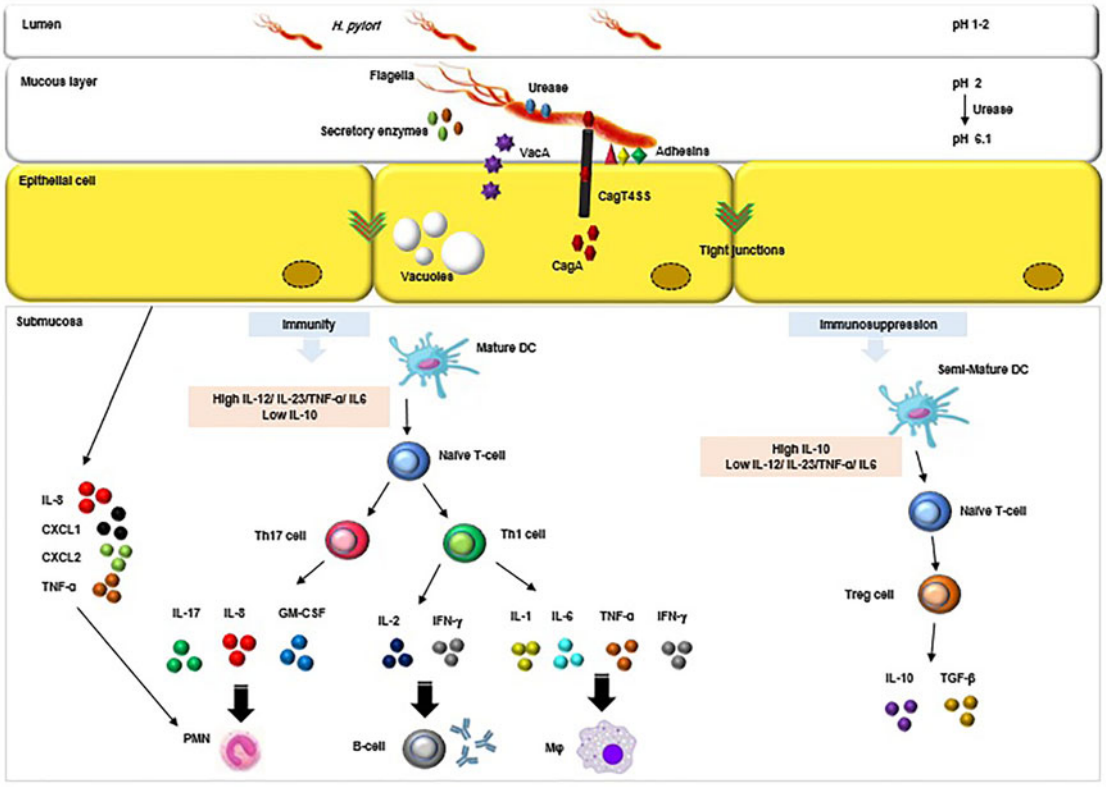


**Figure 5.** The immunological response elicited by *H. pylori* infection. *H. pylori* is genetically highly variable and expresses various virulence factors, including adhesins (BabA/SabA), enzymes (urease), toxins (VacA) and effector proteins (CagA) which are involved in bacterial pathogenesis. The immunity and immunosuppression are the consequences of the maturation status of dendritic cells (DCs), which direct T helper cell differentiation. CagT4SS, Cag type IV secretion system; CagA, cytotoxin-associated gene A; VacA, vacuolating cytotoxin A; BabA, blood group antigen-binding adhesin A; SabA, sialic acid-binding adhesin A; DC, dendritic cell; Mφ, macrophage cell; PMN, polymorphonuclear leukocyte; Treg, regulatory T cell; Th1/17, T helper cell1/17; GM-CSF, granulocyte–macrophage colony-stimulating factor; TNF-α, tumour necrosis factor-α; IFN-γ, interferon-γ; TGF-β, transforming growth factor-beta, CXCL1/2, C-X-C motif chemokine ligand 1/2; IL-1/2/6/8/10/12/17/23, interleukin 1/2/6/8/10/12/17/23.

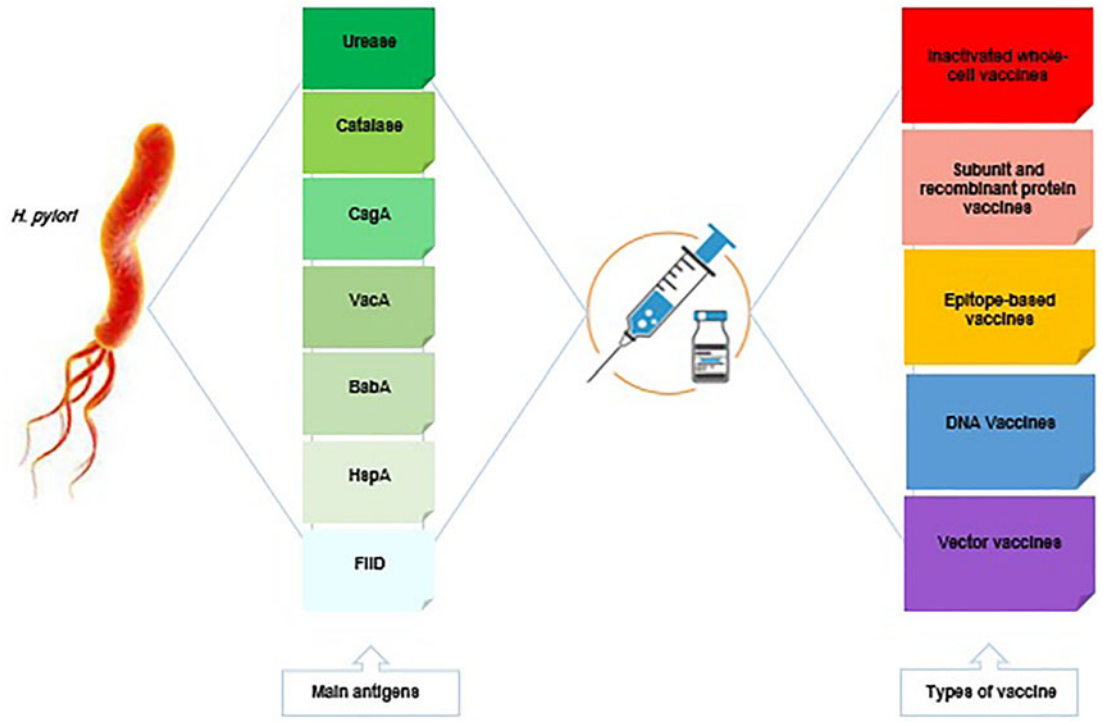


**Figure 6.** Vaccine development against *H. pylori*. CagA, cytotoxin-associated gene A; VacA, vacuolating cytotoxin A; BabA, blood group antigen-binding adhesin A; HspA, heat shock protein A; FliD, Flagellar hook-associated protein 2.

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various immunodominant antigens involved in different aspects of *H. pylori* colonisation and pathogenesis could be a hopeful strategy for the eradication of this insidious bacterium (41).

## Conclusions

*H. pylori* is an uninvited guest in the stomach mucosa of more than 50% of mankind. Variable morphology and expression of numerous heterogenic virulence factors are two intrinsic traits that make *H. pylori* a successful pathogen in the hostile stomach environment. Using various mechanisms, *H. pylori* manages to escape from the innate and adaptive immune responses. In this condition, the immune response cannot eradicate the bacterium and facilitates bacterial colonisation and survival in the gastric mucosa (12, 207, 303). Therefore, immune evasion makes vaccination a challenging issue. In the majority of infected individuals, *H. pylori* and its host are in balance leading to chronic infection without any serious clinical outcomes. However, this balance is not universal, and individuals develop various clinical outcomes from asymptomatic infection to GC. The interaction between the environmental factors (e.g., diet and smoke), host genetic polymorphism (e.g., IL-1β, IL-10, and TNF-α), and the *H. pylori* virulence factors (e.g., CagA and VacA) will determine the clinical outcomes in individuals. So far, a wide range of virulence factors have been attributed to *H. pylori* classified as adhesins (BabA), enzymes (urease), toxins (VacA), and effector proteins (CagA) to influence the host-pathogen interactions. The induction of chronic persistent infection despite a strong host immune response is a unique feature of *H. pylori* pathogenesis. The identification of the host inflammatory pathways that *H. pylori* activates and subverts might help to understand the complicated pathogenesis of *H. pylori*. Therefore, the profound understanding of the exclusive role of *H. pylori* virulence factors in (i) induction of various clinical outcomes and (ii) *H. pylori*–host interaction to subvert the immune response will pave the way for alternative therapies and vaccine development.

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