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REVIEW ARTICLE

Pathogenicity of *Helicobacter pylori* in cancer development and impacts of vaccination

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Abstract

Helicobacter pylori affect around 50% of the population worldwide. More importantly, the gastric infection induced by this bacterium is deemed to be associated with the progression of distal gastric carcinoma and gastric mucosal lymphoma in the human. *H. pylori* infection and its prevalent genotype significantly differ across various geographical regions. Based on numerous virulence factors, *H. pylori* can target different cellular proteins to modulate the variety of inflammatory responses and initiate numerous "hits" on the gastric mucosa. Such reactions lead to serious complications, including gastritis and peptic ulceration, gastric cancer and gastric mucosa-associated lymphoid structure lymphoma. Therefore, *H. pylori* have been considered as the type I carcinogen by the Global Firm for Research on Cancer. During the two past decades, different reports revealed that *H. pylori* possess oncogenic potentials in the gastric mucosa through a complicated interplay between the bacterial factors, various facets, and the environmental factors. Accordingly, numerous signaling pathways could be triggered in the development of gastrointestinal diseases (e.g., gastric cancer). Therefore, the main strategy for the treatment of gastric cancer is controlling the disease far before its onset using preventive/curative vaccination. Increasing the efficiency of vaccines may be achieved by new trials of vaccine modalities, which is used to optimize the cellular immunity. Taken all, *H. pylori* infection may impose severe complications, for resolving of which extensive researches are essential in terms of immune responses to *H. pylori*. We envision that *H. pylori*-mediated diseases can be controlled by advanced vaccines and immunotherapies.

Keywords *Helicobacter pylori* · Virulence factors · Gastric cancer · Host factors · Molecular mechanisms · Vaccination

Introduction

Helicobacter pylori bacterium is one of the most common human infectious agents around the globe. Genetic sequencing evaluation suggested that human and *H. pylori* have been co-evolved for a very long time [1]. Since its discovery in 1982, *H. pylori* bacteria have directly been associated with a range of gastrointestinal conditions [2]. Currently, it is believed that *H. pylori* is the most frequent etiologic agent involved in the infection-associated cancers, which holds 5.5% of the cancer burden in the worldwide [3]. The prevalence of *H. pylori* is dependent on several factors, including geographical region, socioeconomic position, educational level, background and residing setting and lifestyle [4].

Two regular insufferable neoplasms started in the stomach include (i) adenocarcinoma and (ii) lymphoma of gastric, the so-called mucosa-associated lymphoid tissue (MALT). Although the possibility of gastric carcinoma infection has declined in some countries, this disease is considered as the major reason for the cancer-related demise in the world [3]. Unfortunately, many gastric cancers (GCs) are diagnosed in the advanced stages, so that the treatment of these diseases are rarely achieved by surgery and adjuvant therapy approaches [5, 6]. Gastric carcinomas and MALT lymphomas seem to occur in a setting of chronic gastric inflammation [7]. However, in the two past decades, the most

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common reason for gastritis was shown to be induced by the consistent *H. pylori* infection. It should be noted that almost 70% of global gastric cancer and gastric MALT lymphomas incidences are happening by the previous infection with *H. pylori* [6, 8].

Altogether, among different diseases that strongly linked to *H. pylori* infection, gastrointestinal malignancies remain as one of the most life-threatening ailment that require a better understanding of the molecular mechanism(s) involved with the initiation and progression of the disease. Hence, in this review, we present some important mechanistic insights into the involvement of *H. pylori* in gastric carcinogenesis and the impacts of passive and active vaccinations.

A glance at the pathogenesis of *H. pylori*

H. pylori is a Gram-negative spiral-shaped bacterium (Fig. 1) acquired often in the human infancy that can induce chronic gastric inflammation during human life, which is also the most crucial riskiness factor for gastric malignancies [9]. The impacts of *H. pylori* infection on the gastric malignancies might depend on the anatomic location [10]. However, gastroesophageal junction cancer might be associated with either *H. pylori* infection or Barrett's esophagus [11]. Therefore, the abolition of *H. pylori* has become a typical treatment modality in people with the gastric MALT lymphoma. Because *H. pylori* do not adhere effectively to the abdominal mucosal cells, linking this infection to the intestinal type gastric cancer may not be obvious when the intestinal metaplasia dominates the gastric topography. For the same reasons, serum antibody levels against *H. pylori* antigens decrease during the development of gastric cancer [12]. Although *H. pylori* mostly adhere to the epithelial cells of the stomach, it may also colonize at the proximal duodenum resulting in a possible transformation of gastric tissue (the so-called metaplasia).

Accordingly, it has been articulated that the intracellular presence of *H. pylori* may promote its persistence, resulting in the induction of an inadvertent antibiotic resistance [13]. *H. pylori* utilize its very effective enzymatic pieces of machinery (e.g., urease) to buffer the gastric environment with pH 1–2 acidity [14]. Furthermore, the survival of

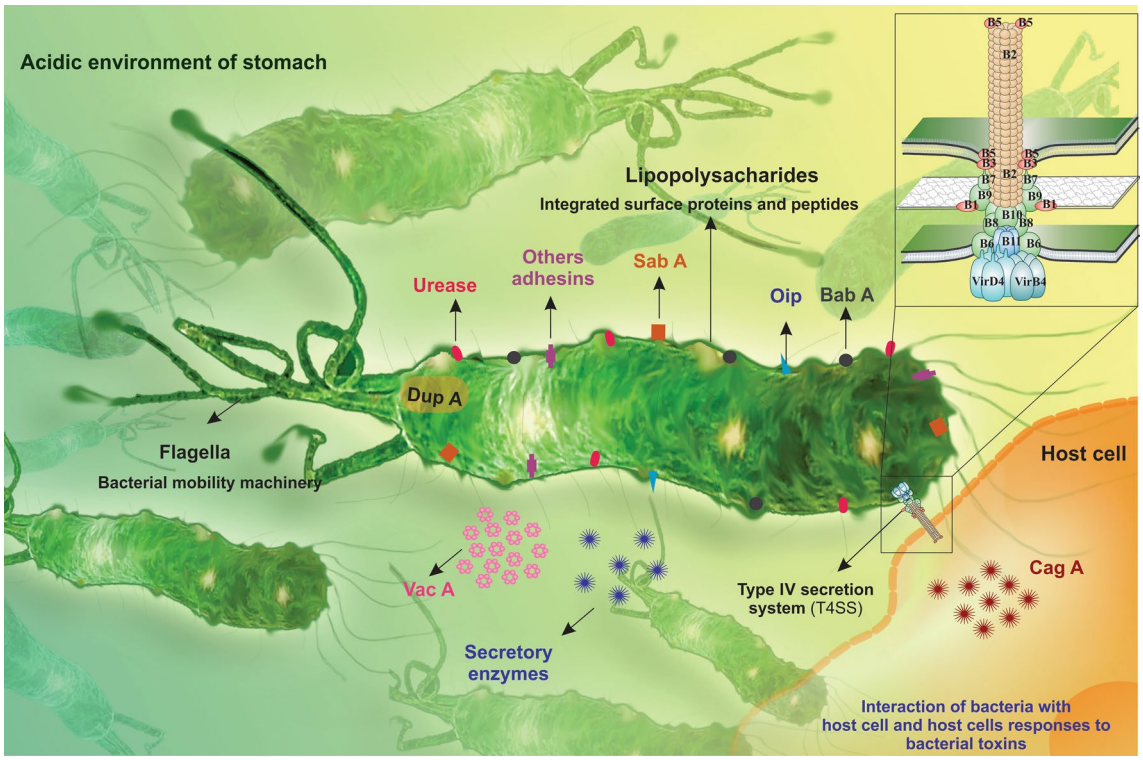

Fig. 1 *Helicobacter pylori* structure and its infection mechanism. Various bacterial entities (e.g., toxins and enzymes) are involved in the interaction of bacteria with the host cells and its evasion from the immune system surveillance. *Flagella* gives motility and enables the bacterium to grow under the mucosal membrane. *LPS* lipopolysaccharides and membrane proteins adhere to the host cell receptors. *Urease* enzyme is used to combat the acidic environment of the stomach by producing ammonia. *VacA* exotoxin causes injury to the mucosal membrane. *T4SS* Type IV secretion system that uses a pillus to inject effectors (inset). *CagA* causes actin remodeling and inhibits apoptosis. *Outer proteins (BabA, Oip, SabA, Others adhesins)* adhere to the host cells

bacteria is facilitated by the helical morphology and unipolar flagella permitting movement within the gastric mucous layer via overlaying among and/or within the gastric epithelial cells (Fig. 1) [15].

Genomics of *H. pylori*

Variations of the *H. pylori* genome is significantly associated with the migration trends of human populations, which connecting the geographical dissemination of the microorganism leading to the emergence of mankind life [16]. *H. pylori* genome was sequenced in 1997 [17] and since then several full genome sequences (at least 7) have been reported [17–22]. Generally, the *H. pylori* genome comprises about 1.6 megabases, encoding approximately 1500 predicted open reading frames (ORFs) and about 20–30% genomic variation among different strains. This issue is relatively high percentage of bacterial species, resultant from the high spontaneous mutation rate and recombination frequency within the microorganism genome [23]. Several variable regions within the *H. pylori* genome have already been identified, including "plasticity zone" and "cytotoxin-associated gene" (*Cag*) pathogenicity island. The *Cag* island encodes a few structural proteins that required for assembling four secretion systems, effective on the translocation of the *H. pylori* products (e.g., immune dominant 120–145 kDa *CagA* protein) [24] within the host gastric epithelial cells [25]. The *H. pylori* genome also encodes several adhesion proteins that are important for ensuring tight contact between *H. pylori* and gastric epithelial cells. These proteins include the blood group antigen binding adhesin (*BabA*) and the sialic acid binding adhesin, *SabA*. *VacA* gene of *H. pylori* encodes a multimeric vacuolating secretory cytotoxin (88 kDa), which is effective for developing the intracellular vacuoles in the gastric and different epithelial cells [26]. This gene is conserved among all *H. pylori* strains. Nevertheless, the gene exhibits a high level of genetic variation within regions that encode the signal sequence, intermediate factor, and the middle portion of the *VacA* protein [27]. The recent evaluation of the transcriptome of *H. pylori* strain 26,695 has revealed that the simultaneous presence of sense and antisense transcripts from common RNA sequences within the organism [28], introducing another level of complexity of the genome. Multiple RNAs forms such as non-coding RNAs were also reported within the *H. pylori*.

H. pylori pathogenesis in GC

H. pylori produce many different virulence factors that may dysregulate the host intracellular signaling mechanism(s) and promote the neoplastic transformation [29]. Among those, *CagA* and its pathogenicity region (*Cag* PAI), and *VacA* (vacuolating cytotoxin A) are deemed as the significant pathogenic factors, which will be discussed in the following sections (Fig. 2) [24].

Impacts of *CagA* in GC

The *Cag* PAI is a 40 kb locus comprising 27–31 genes. A few genes within this locus encode the *CagA* protein and the *Cag* type IV secretion process (T4SS) [30]. Of these, the T4SS forms a syringe-like pilus structure, through which the *CagA* protein can be injected into the host cells, leading to the modulation of cellular processes in the favor of the pathogenic activity of an invader. For such phenomena, the ectodomain of a5b1 integrin seems to be a vital step for the translocation of *CagA* into the host cells [31]. Subsequently, *CagA* binds to the internal surface of the cell membrane and undergoes tyrosine phosphorylation at glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) site by Src family kinases. It should be noted that the phosphorylated and unphosphorylated *CagA* interact with numerous host proteins, resulting in the activation of some downstream signaling pathways, including the Ras/mitogen-activated protein kinase (MEK), extracellular signal-regulated kinase (ERK) [32, 33], nuclear element κB (NF-κB), and β-catenin pathways. These biological functions appear to enhance the proliferative ability of the gastric epithelial cells (Fig. 2) [34].

Impacts of *VacA* in GC

H. pylori secrete the vacuolating cytotoxin (*VacA*) via a type V auto transport release system [24]. *VacA* is an 88 kDa, constituting p33 and p55 subunits, in which the p33 protein (N-terminal, 33 kDa) forms an inner channel for the chloride transportation and the p55 protein (C-terminal, 55 kDa) is responsible for the presentation of toxin into the host cells [35]. *VacA* has several biological activities, and it can bind to a variety of cells. After the internalization, it can induce intense vacuolation by the accumulation of large vesicles existed in both early and late endosomes. *VacA* can be transferred to the mitochondria, in which it causes the dissipation of mitochondrial transmembrane potential ($\Delta\Psi_m$), discharge of cytochrome c, and the activation of pro-apoptotic factor Bcl-2 associated X protein (Bax). All these proteins can eventually involve in the apoptosis [36]. During *VacA*-induced mitochondria perturbation, the activation of dynamin-related protein 1 (DRP1) may play a critical role because the inhibition of DRP1-dependent mitochondria fission within the *VacA*-intoxicated cells was shown to inhibit the activation of Bax and mitochondrial outer membrane permeabilization

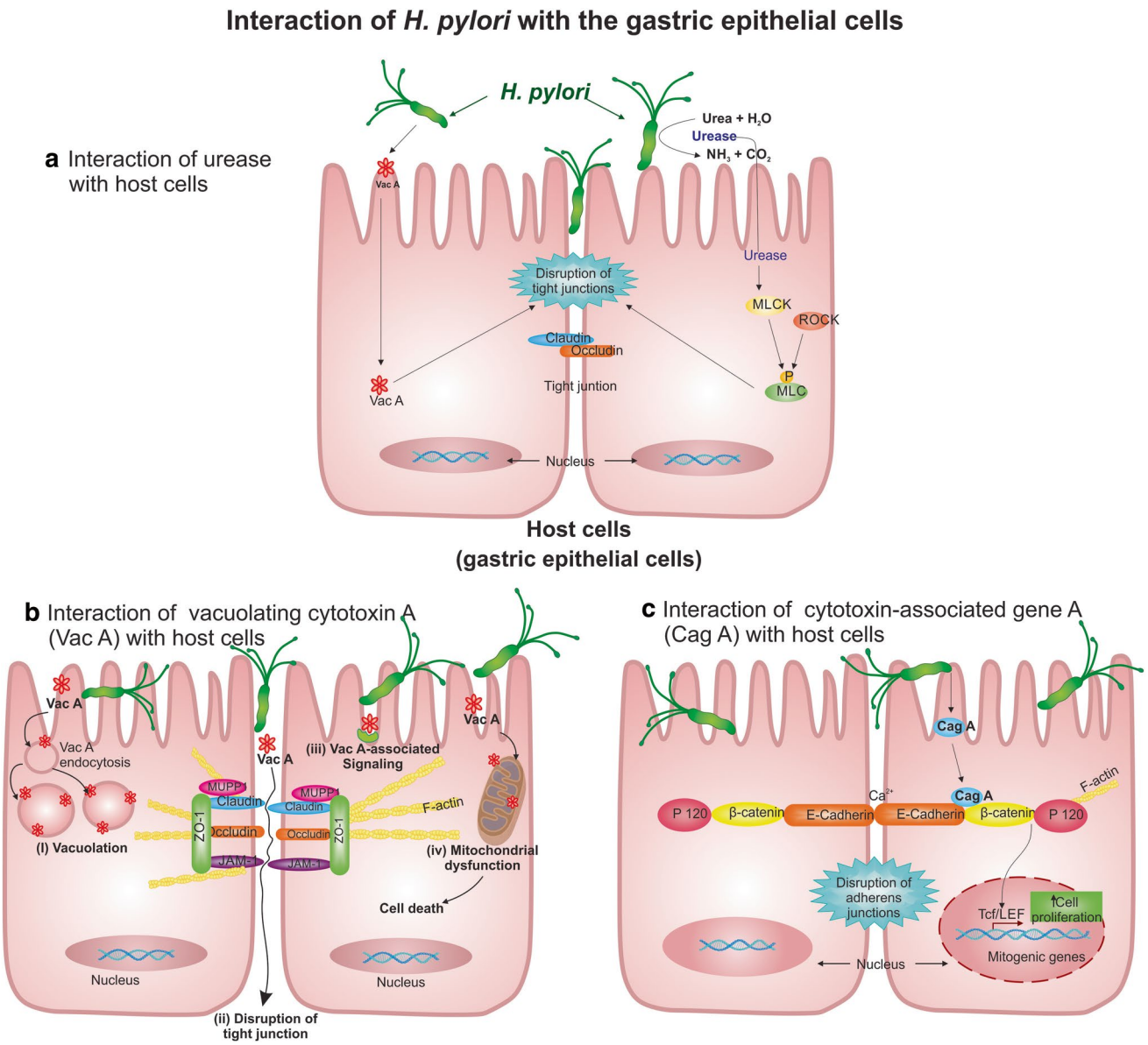

Fig. 2 Dysregulation of the apical-junctional complex by *H. pylori*. **a** Released and imported urease can phosphorylate MLC by MLCK kinase, leading to the disruption of tight junctions between the stomach cells. **b** Vacuolating cytotoxin A (VacA) is secreted by the bacteria, which can then bind to host cells. Once internalized by the host gastric epithelial cells, VacA can induce a severe vacuolation—seen as the accumulation of large vesicles similar to early and late endosomes, and early lysosomes. The development of "vacuoles" has been attributed to the formation of VacA anion-selective channels in membranes. Besides, the p33 subunit of VacA can enter into the mitochondria and disrupt their function. Moreover, VacA by effecting on the tight junction elements such as occludin, claudin and JAM-1 can disrupt the junctions between the stomach cells. **c** After importing the *CagA* into the stomach cells, it can physically interact with the E-cadherin, releasing of and importing β-catenin into the nucleus. *H. pylori* can induce the nuclear translocation of p 120 protein, which can increase the releasing level of β-catenin. In the nucleus, β-catenin can induce the accumulation of the tcf/LEF and subsequently the highly increasing the cell proliferation

(MOMP) and also prevent the death of intoxicated cells [37]. Moreover, *VacA* can affect the restricted connections of the epithelial cells and prevent T lymphocyte activation and expansion in the lamina propria. It seems that the disruption of the autophagy could be considered as another mechanism by which *VacA* causes gastric inflammation, and hence, contributes to gastric carcinogenesis (Fig. 2) [38, 39].

H. pylori responses and its role in the induction of GC

H. pylori infection and the resultant chronic inflammation in the gastric mucosa is believed to be the major part of the initiation, development, and progression of GC. In fact, *H. pylori* bacteria stimulate an inflammatory response both in the gastric epithelial cells and the immune cells recruited to the site of infection through multiple mechanisms [40]. It has been shown that the *H. pylori* infection upregulates the functional expression of several pro-inflammatory cytokines such as interleukin (IL) 1, IL-6, IL-8, tumor necrosis factor α (TNF-α), NF-κp, and also regulates the activation of regular T cells [40]. These cytokines, particularly NF-κB, are key mediators of gastric pathophysiology and may perform important roles in the development of gastric inflammation and cancer (Fig. 3).

Role of CSCs in *H. pylori*-induced gastric inflammation and carcinogenesis

Cancer stem cells (CSCs) are considered as a special set of cells with the self-renew ability for the differentiation to mature tumor cells [41]. Recently, it is thought that CSCs perform a pivotal role in the development of several cancers such as GC [42]. Although the origin of the gastric CSCs is not absolutely clear, it is believed that they originate from the segregated gastric epithelial cells,

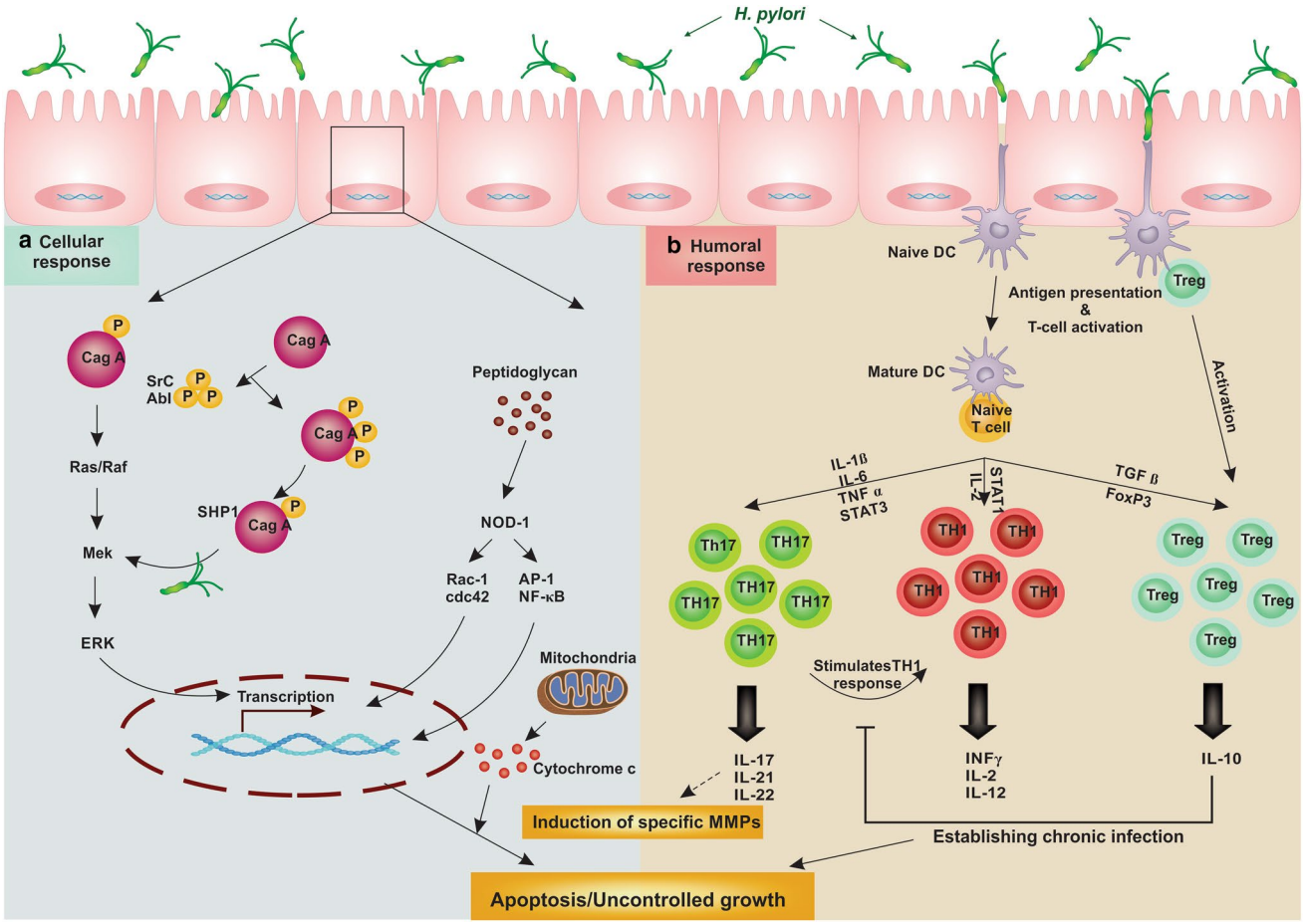

Fig. 3 Schematic view of *H. pylori* infection and the induction of matrix metalloproteinases (MMPs) involved with both cellular and humoral components. **a** Cellular responses. During the infection of *H. pylori, CagA* with type IV Secretion System (TSSIV) enter into the gastric epithelial cells. *CagA* phosphorylation occurs by host's *Src/Abl* kinases and the phosphorylated CagA activates a series of signaling molecules such as inflammatory cytokines, ROS, MMPs, leading the aberrant cellular function. **b** Humoral responses. *H. pylori* prime the host immune system by various lymphocyte subsets through dendritic cells (DC)-mediated antigen presentation to the naive T cell. Under the influence of specific cytokines and foreign antigens, the naive T cells start switching and differentiate into the effector T subtypes via signature transcription factors. TH17 and TH1 promote the inflammatory response while Treg arrests the reactions by secreting immunosuppressive cytokines; and thereby, maintaining *H. pylori* inside the gastric mucosa. TH17 stimulates MMPs through IL-17 and IL-21

local progenitor cells in the gastric mucosa, or bone marrow-derived cells (BMDCs) [43]. Some in vivo animal studies have demonstrated that the chronic inflammation could induce gastric CSCs, resulting in *H. pylori*-induced GC [44]. Intriguingly, some virulence factors of *H. pylori* (e.g., CagA protein, T4SS or VacA protein) might not be involved in the mobilization of gastric CSCs, but rather, the certain undiscovered virulence factors and cytokines secreted by contaminated epithelial cells (e.g., TNF-α) could be involved in this process [44]. The role of BMDCs in *H. pylori*-induced gastric carcinogenesis has recently been reported, showing that the strains of *H. pylori* were able to recruit bone marrow stem cells to the gastric mucosa via different capacities, upon which they could turn into the gastric glands with the possibility to evolve towards metaplasia and dysplasia [45].

Mechanisms of *H. pylori*-induced gastric carcinogenesis

The chronic inflammation is stimulated by the persistent *H. pylori* infection, which can create a permissive microenvironment with the plethora of inflammatory cytokines as well as reactive oxygen and nitrogen species (ROS and RNS) that have the potential to induce cellular damage and mutagenesis [46]. Accelerated cell turnover in such microenvironment can result in (a) the emergence of cell lineages which are not normally found in the stomach (gastric metaplasia) and in a small part of humans infected chronically by *H. pylori*, and (b) the development of neoplastic clones under the pressure of accelerated DNA replication [47]. As such, the chronic inflammatory state is deemed to be the feature of several common individual malignancies, especially in the gastrointestinal tract. Other such examples include chronic acid reflux esophagitis (predisposing to Barrett's metaplasia and esophageal adenocarcinomas), chronic viral hepatitis (increasing the danger of hepatocellular cancer) and the chronic colonic inflammation of the inflammatory bowel diseases (IBD) that are associated with the increased risk of colon cancer [48].

Some investigations focusing on pro-inflammatory immune responses against *H. pylori* infection have mainly been designed based on in vitro models using gastric epithelial AGS cells. Further, it is critical to define the modulating mechanisms of the antigen presenting cells such as dendritic cells (DCs) as well as B and T lymphocytes by *H. pylori*. Given the interactions between pathogen and gut-associated immune cells, it is envisioned that DCs play a major role, through Toll-like receptors (TLRs), in the regulation of the responses of the adaptive immune against *H. pylori* [49] (Fig. 3).

H. pylori-induced changes in epithelial gene expression and regulation

Of numerous alterations induced by *H. pylori* in the gastric epithelial cells, the differential expression of various gene clusters plays vital roles in promoting gastric epithelial cell transformation [50]. Although TP53 mutation is one of the common molecular hallmarks of various malignancies, there are inconsistent studies about the regulation of wild-type p53 appearance by *H. pylori* during the pre-neoplastic stages in GC [50]. Nevertheless, the p73, a homologous of p53 protein, has recently been shown to be highly responsive to *H. pylori*, which seems to be much more important than p53 in regulating apoptotic phenomena within the gastric epithelial cells [51].

H. pylori infection has a causal role in the induction of specific alterations on the DNA methylation patterns in the gastric mucosa of *H. pylori*-infected patients and GC cell lines [52]. Of these genes that specifically methylated by *H. pylori* infection, the E-cadherin (CDH1) has a specific importance in the diffuse type of gastric carcinogenesis. In fact, germline mutations in CDH1 are responsible for the syndrome of hereditary diffuse GC and commonly acquire in the sporadic diffuse type of GC [53]. It should be noted that the methylation of the E-cadherin promoter is reverted to the normal state after the eradication of *H. pylori* [54]. *H. pylori* infection also causes hypermethylation leading to a decrease in the expression of DNA repair protein O-6-methylguanine-DNA methyltransferase. The latter enzyme is a biomolecule, which is normally involved in the prevention of cytosine: guanine to adenine: thymine change mutations, and thus, DNA replication fidelity [55].

Several groups have recently analyzed the microRNA signatures of the *H. pylori* infection and showed that the dysregulation of miRNA expression could be mechanistically linked between the *H. pylori* infection and the development of gastric malignancies [56]. Furthermore, a study of the differential expression of microRNAs between *H. pylori*-positive and negative patients showed that 14 of 30 miRNAs after *H. pylori* eradication were repaired [57]. Further, the upregulation of certain miRNAs such as the members of let-7 family is affected in the presence of the Cag pathogenicity island. However, miR21 and miR155 could be upregulated during the occurrence of *H. pylori* infection, which may provide some insights about the significance of these markers in vivo [58–60].

Loss of gastric acidity

H. pylori bacteria are one of the bacterial species that can efficiently survive in an extreme condition (pH 2–3) of the gastric lumen. The resident bacteria in the oral cavity and gastrointestinal regions can be colonized in the stomach if the gastric pH reaches to pH 7. The chronic gastric inflammation may be predisposed to GC through atrophy of gastric glands due to the lack of the specialized acid-secreting parietal cells [61]. In the state of hypochlorhydria, the colonization and growth of non-*Helicobacter* species may generate carcinogenic and potentially genotoxic nitrosamines [62]. It should be noted that the interplay between *H. pylori* infection, gastric acid secretion and clinical outcome is complicated, which is dependent on a large region within the stomach with maximal *H. pylori* infection. For instance, despite being infected by *H. pylori*, individuals who involved with the duodenal ulcers showed high acid secretory rates, while they had a very small chance of developing GC [63]. Such discrepancy seems to be explicable on the foundation of *H. pylori* infection in such patients being solely confined to the gastric antrum rather than other regions. Such infection may result in the depletion of the somatostatin secreting cells in the region and eliciting a subsequent exaggerated release of the acid secretory hormone gastrin from the particular antral gastrin-secreting neuroendocrine cells. When the proximal stomach has not infected with *H. pylori*, in duodenal ulcer patients, the high levels of gastrin can stimulate the healthy parietal cells of the proximal stomach towards hypersecretion gastric acid, causing inevitable ulcerative damages in the proximal duodenum [64]. Although some patients have high levels of gastrin, their parietal cells present an intriguing hyper-functional environment of intense proximal stomach inflammation and consequently glandular atrophy of *H. pylori*-induced inflammation [65].

Role of oxidative stress and DNA damage in *H. pylori*-induced gastric inflammation and carcinogenesis

The pathogenesis of *H. pylori*-associated gastric carcinogenesis appears to be associated with the generation of intracellular ROS RNS in the human stomach, as well as the oxidative stress and DNA damages (e.g., p53) [66, 67]. It is demonstrated that *H. pylori* can induce the generation of ROS and RNS in the host gastric epithelial cells and inflammatory cells (e.g., neutrophils) [68], indicating their important roles in the gastric carcinogenesis.

Gastric immune response to infection

In the absence of *H. pylori* antigenic stimulation, the stomach appears to act as a relatively quiescent organ with little evidence of immunologic activity. Further, an oral immunization that supports the trafficking and migration of antigens into the mucosal organs of antigen-specific T cells and IgA B cells may result in originating in the gut-associated lymphoid tissues (GALT). So that, the uninfected stomach is segregated from the continuous entry of lymphocytes into the mucosal sites [69, 70]. These findings indicate a paucity of local gastric cytokines and chemokines involved in guiding integrin expression, leukocyte homing, and an influx in the absence of *H. pylori*-driven inflammation. Early events during binding *H. pylori* to the gastric epithelial cells are mediated by the interaction of epithelial cell glycoconjugate and integrin receptors with their cognate *H. pylori* ligands [71–74]. Adhesion reaction induces the translocation of *H. pylori* protein antigens into the epithelial cells by type IV secretion [75] and the focal reorganization of cytoskeletal proteins into the membrane pedestals [76]. Tyrosine phosphorylation of the host proteins leads to the activation of NF-κB transcription factor than promoting the production of inflammatory cytokines and chemokines [77, 78]. Accordingly, gastric biopsies from the infected subjects exhibit the increased levels of several factors, including interleukin-1β (IL-1β), IL-6, IL-8, IL-12, tumor necrosis factor alpha (TNF-α), growth-related oncogene, monocyte chemotactic protein-1, macrophage inflammatory protein-1 alpha, and regulated-upon-activation T expressed and secreted (RANTES) chemokines [79]. The chemical gradients created by these molecules can be harmonized the expression of cell adhesion receptor–ligand pairs and favor the leukocyte recruitment, accumulation, and activation (Fig. 3).

It is clear that during the infection of gastric tissues, *H. pylori* upregulates the expression of the CD11b/CD18 integrin and its receptor and intercellular-adhesion molecule-1 (ICAM-1; CD54) used for the leukocyte transmigration into inflammatory sites [80–82]. *H. pylori* can also increase the expression of CD80 and CD86 in gastric epithelial cells [83] required for the costimulation T cells and the upregulation of class II major histocompatibility complex (MHC) in vivo [84, 85]. Importantly, the class II of MHC heterodimer may itself act as a receptor for *H. pylori* [86].

Immune evasion strategies employed by *H. pylori*

To explain its persistence in human populations, *H. pylori* has been suggested either to tolerize the host from mounting a protective immune response or to interfere with the

immune responses that would otherwise result in its elimination [87]. While the carriage of the *H. pylori* does not induce peripheral tolerance, several studies indicated the ability of *H. pylori* to downregulate the T-cell proliferation [88] and IL-15 transcription [89] and also to restrict the cognate interactions in the T-cell activation through the perturbation of endocytosis and antigen processing [90]. Recent findings showed that the persistence of *H. pylori* might also be related to its capacity to inhibit T cells by the induction of apoptosis through Cag pathogenicity island (PAI) and the expression of Fas ligand and evade the immunosurveillance [91]. *H. pylori* can polarize the host cells and T cells and alter their responses. Given that the *H. pylori* infection is dominated by the regulatory T cells and T helper 1 (Th1) cells through induced Th17 and the expression of IL-17 and IFN-γ [92, 93], its immunization may be observed as a type of Th1-dominant response that is in favor of the bacterial growth and progression.

Vaccines

Vaccines have been developed against a large number of infectious and non-infectious diseases [94]. Early attempts focused on the recombinant urease showed some promising outcomes in animals, nevertheless, some subsequent clinical trials were hampered by a number of certain side effects of mucosal adjuvants [95]. More recently, an intramuscularly administered trivalent vaccine (recombinant CagA, VacA, and neutrophil-activating protein) was developed, while unfortunately the antigens were recognized by the host's cellular and humoral immune systems, causing no immunity in a challenged model [96]. Chen et al. synthesized an *H. pylori* oipA DNA construct, as a therapeutic vaccine, that was delivered by attenuated *Salmonella typhimurium* in the C57BL/6 mouse model with *H. pylori* strain SS1 infection [97]. To increase the expression level, the *oipA* gene was codon-optimized for the mammalian cell systems, resulting in a 2-log reduction of *H. pylori* colonization with sterilizing immunity achieved in three out of 10 mice. The LPS of *H. pylori* is relatively nontoxic but may promote autoimmune responses. Considering the potential of polysaccharide-based conjugate vaccines, Altman et al. chemically modulated the LPS of *H. pylori* by delipidation and conjugation processes to enhance the immunogenicity [98]. Prophylactically administering of the oipA antigen induced enhanced antibody responses and a modest reduction in gastric *H. pylori* loading. Two groups of tested *H. pylori* antioxidant proteins in the mouse models demonstrated the partial protection for both alkyl hydroperoxide reductase (AhpC) [99] and a trivalent superoxide dismutase/catalase/thiol peroxidase preparation [100]. The *AhpC* was found to be beneficial when administered subcutaneously with alum, while the trivalent vaccine was successful intranasally with the cholera toxin. Moreover, mannosylation could generally improve the antigen presentation, nonetheless, the protection afforded by mannosylated *AhpC* was no better than that of the native protein [99]. Recently, based on the relative immunodominance of *H. pylori*, the Lpp20 outer membrane lipoprotein in the immunized rabbit antiserum was used to prime BALB/c mice with the recombinant Lpp20 [101]. Then, splenic T-cell responses were analyzed to eight peptides predicted in silico as Lpp20 epitopes. Two of these epitopes showed immunogenicity through the proliferation and cytokine secretion assays. Furthermore, some researchers used restricted HLA and evaluated their immunogenicity effects. Based on the results obtained from murine studies of a multi-T-cell epitope construct against urease B, dominant UreB T-cell epitopes were identified in two *H. pylori*-infected patients [102]. Each subject revealed the dominant HLA-restricted T-cell responses to different regions of UreB identified by the peptide stimulation in vitro. However, the applicability and practicality of this approach and the development of haplotype-specific vaccine remain to be determined. The same group [103] used a multi-T-cell epitope pseudo protein containing 17 putative *HpaA, UreB*, and *CagA* epitopes. Once administered prophylactically subcutaneously in BALB/c mice, the colonization was decreased by 1–2 logs. Despite the modestly improving parameters of humoral and cell-mediated immunity, none of the four tested adjuvants could significantly enhance the vaccine efficacy. It was reported that a single epitope of urease A, given intragastrically as a 20-mer peptide with cholera toxin B as an adjuvant, achieved a 1-log reduction in BALB/c mice administered either prophylactically or therapeutically [104]. Identifying the optimal adjuvant/delivery strategy is critical for the clinical trials. Given that cholera toxin and *Escherichia coli* LT antigen can induce diarrhea in humans, a recently developed LT double mutant (R192G/L211A) was tested via the sublingual or intragastric route together with *H. pylori* lysate in mice [105]. The LT mutant was similar to the cholera toxin in terms of protective immune responses and efficacy. An alternate adjuvant strategy is the use of an engineered chimeric flagellin (*H. pylori*/*E. coli*) to activate TLR5. The prophylactically administered vaccine (as boosts given with alum) was reported to significantly reduce the *H. pylori* DNA levels in association with enhanced serum IgG antibody levels [106]. Finally, because *H. suis* is a significant cause of gastric ulcers in pigs, a BALB/c mouse vaccine model was developed against this disease. Whole *H. suis* lysate or recombinant *UreB*, but not *rNapA,*

showed promise in terms of the bacterial colonization when administered prophylactically [107].

Retrospective and new considerations in *H. pylori* vaccine development

The results of the clinical studies (Table 1) performed to date present a re-evaluation of the understanding of *H pylori* immunity. Although it must be acknowledged that the mouse model is extremely useful for helping to define differences in the nature of the immune responses to infection and immunization, it may lack predictive ability when designing an efficacious vaccine. Most studies defined protection as a significant reduction in bacterial load, with only occasional reports of sterilizing immunity [108, 109]. Additional evidence revealed that the levels of protection observed in mice might not reflect its use in the human. Based on the data obtained from the primate studies (e.g., *rhesus macaques* harboring native *H. pylori* infections), one may deduce that in a model of indigenous *H. pylori* infection, vaccines similar to those tested on mice are much less efficacious [110, 111]. In fact, the host response to *H. pylori* is actively suppressed by regulatory T (Treg) cells and IL-10-producing Tr1 cells [49, 112]. In vitro lymphocyte recall assays on infected and non-infected subjects demonstrate comparable responses to *H. pylori* antigen [112, 113]. Further, depletion of CD25hi T cells led to significant activity in the T cells isolated from *H. pylori*-infected donors [114]. Subsequent experiments have documented that the presence of Treg cells in the infected human stomach might cause a blockage or suppression of Treg cells, and hence, significantly increasing the activity of T-helper cells and gastric inflammation and markedly reducing/eliminating the bacterial load from the stomach [49, 115, 116]. The host gastrointestinal tract inherently suppresses the immune responses to commensal bacteria, thus future strategies might incorporate the mechanisms of limiting Treg cells activity or preferentially activating proinflammatory T cells that can overcome the activity of Treg cells. In that light, the administration of IL-12 to *H. felis*-infected mice was sufficient to achieve the eradication of the bacteria in the absence of immunization [117]. Finally, although the results have yet to be published, a large-scale phase III of the clinical trial was completed in China to test a prophylactic oral vaccine against the natural acquisition of *H. pylori* [118]. The vaccine was tested on children aged 6–15 years who were negative in terms of *H. pylori*. The oral vaccine contained the urease B protein subunit, while additional details remain unknown. It was administered in three 15-mg immunization doses, and the children were monitored to determine the rate of natural *H. pylori* infection. Studies in mice indicate that antibodies are sufficient to prevent the infection when present at a challenge sufficiently [119]. Recently, a novel oral vaccine has been constructed against the *H. pylori* infection in the children. On the basis of the phase III data, the researchers claimed the vaccine as a safe and well immunogenic treatment modality that could prevent the *H. pylori* infection with a high rate (up to 71·8%) [120]. To date, almost twenty US patents have been submitted about the different aspects of *H. pylori* infection from 1997 to 2015 years.

New strategies about *H. pylori* vaccine construction

Recently, some researchers have focused on the recombinant vaccines with multiple T- and/or B-cell epitopes against *H. pylori* infection. Multi-epitope vaccines that generally composed of $CD4^+$ and $CD8^+$ epitopes have been examined due to their safety, stability, cost-effective production, and high specificity. However, the main drawback of this approach is the low immunogenicity that is also observed in the oral vaccines [124].

Identifying and using the protective antigens and virulence factors is another approach for designing vaccines

Table 1 Clinical trials for vaccine efficacy against *H. pylori*

References	Year	Route	Antigens	Adjuvant	Timing	Challenge	Result
[121]	1999	Oral	Urease	LT mutant	Therapeutic	Natural	Significant reduction in bacterial load in some vaccine groups
[122]	2001	Oral	Whole cell	LT mutant	Therapeutic	Natural	No clearance
[123]	2008	Oral	Urease or Hp0231	*Salmonella enterica* serovar Typhi Ty21a	Prophylactic	Experimental	Some clearance in both vaccine and control groups
[96]	2012	Intramuscular	CagA VacA Nap	Alum	Prophylactic	Experimental	Clearance equivalent between vaccine and controls groups
[118]	2014	Oral	Urease	Undisclosed	Prophylactic	Natural	Efficacy, 72%
[120]	2015	Oral	Urease	Undisclosed	Prophylactic	Experimental	Significant prevention of the infection in the children

against developing cancers and *H. pylori* infection. Different virulence factors have been used for constructing high effective vaccines against *H. pylori*, including urease (*UreA* and *UreB*), vacuolating cytotoxin (*VacA*), neutrophil-activating protein (*NapA*), *CagA*, heat shock proteins (*Hsps*), and different types of outer membrane protein (*Omps*) [125, 126]. A chimeric vaccine, consisting of *UreA* and *UreB*, was constructed and expressed in *E. coli*. The recombinant protein was purified and used for intragastric vaccination of Mongolian gerbils [127] and C57BL/6 mice [128], which showed partial inhibition of *H. pylori* infection. Regarding the limited success of intragastric vaccination, some researchers have focused on new platforms for an oral administration of the vaccines, including a vaccine expressed in *Lactococcus lactis* NZ9000, and the spores of *Bacillus subtilis*. Because the bacteria show very low survival in the gastrointestinal tract, the antigen-based vaccines, which was expressed cytoplasmically, could be released in this tract resulting in the minimization of the *H. pylori* infection [125].

Microalgae, as a group of photosynthetic microorganisms, have unique characteristics (fast growth rate and simple and cost-effective cultivation procedure) [129] and can be used as edible vaccines. The cell-wall polysaccharides of different microalgae, which might present a natural encapsulation for the recombinant proteins and protect them within the harsh conditions of the stomach and intestine and act as an effective adjuvant for boosting the immune system [130, 131].

Conclusion

During *H. pylori*-induced chronic inflammation and subsequent carcinogenesis, various bacterial, host, and environmental factors may be associated in the emergence of inflammation and progression of the disease. While having *H. pylori* in over 50% of the world's population, only 2% of the infected cases develop GC and fewer develop a MALT lymphoma. Given such variable threat of *H. pylori* infection-mediated malignancy, what are the important factors or co-factors involved in dictating which individuals with *H. Pylori* infection will undergo *H. pylori*-induced gastric transformation? Some of the variability in outcome can be correlated with the bacterial strain specificity, host genetic susceptibility, and the type of immune response elicited in the infected host.

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Compliance with ethical standards

Ethical approval There is none to be declared.

Conflict of interest The authors declare no competing interests.

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