Microb Health Dis 2021; 3: e537

REVIEW – *HELICOBACTER:* INFLAMMATION, IMMUNITY AND VACCINES

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Dedicated to John L. Telford, the discoverer of Helicobacter pylori cytotoxin VacA, a great scientist, a great man, who died on 27 December 2020 at the age of 70.

Abstract: *Helicobacter pylori* is a bacterium that colonizes the human stomach and is the risk factor for severe gastroduodenal diseases, such as gastric cancer, gastric lymphoma, autoimmune gastritis and peptic ulcer. *H. pylori* activates Nod-like and Toll-like receptors, and usually promotes gastric T-helper 1/17 (Th1/Th17) immune responses. Several bacterial factors, such as CagA, VacA, HP-NAP, the lipoprotein HP1454, and HP0175 can shape Th1/Th17 response by regulating T-cell receptor signaling. However, the host immune response to the infection is ineffective, the bacterium persists, and the inflammation continues for decades. Altogether the investigations performed so far highlight the crucial roles played by both *H. pylori* and the immunopathological responses of the host in the pathogenesis of gastric cancer, gastric MALT lymphoma, and gastric autoimmunity. It also suggests the strong and urgent needing for an anti-*H. pylori* vaccine in order to prevent both the infection and the severe diseases due to *H. pylori.* The present article is a review of the most relevant literature on inflammation, immunity, and vaccines against *H. pylori,* published in the middle of the COVID-19 war between April 2020 and March 2021.

Keywords: Innate immunity, T helper cells, Th1, Th2, Th17, Treg, cytokines, Vaccine, *Helicobacter pylori*, Gastric cancer, Gastric lymphoma, SARS-CoV-2, COVID-19.

INTRODUCTION

Helicobacter pylori chronically infects the stomach of half of the human population worldwide and represents the major cause of gastroduodenal diseases, such as gastric cancer, gastric B-cell lymphoma, peptic ulcer, and gastric autoimmunity. Here, we present an overview of the major findings on the host response to *H. pylori* published between April 2020 and March 2021.

HELICOBACTER PYLORI, INFLAMMATION AND INNATE IMMUNITY

The innate immune system represents the first line of host defense and is crucial to the initiation of inflammation and the adaptive immune response observed in *H. pylori* infection.

Interplay Between Gastric Epithelial Cells, Immune Cells and Gastric Cancer

The epithelial barrier is the first point of contact with *H. pylori*. The evidence that the bacterium adheres to gastric epithelial cells and that it can also invade and proliferate inside them, support the notion that the interaction of the bacterium with this front-line defense plays an important role in immune escape, chronic infection, and induction of gastric pathologies, including gastric cancer (GC), which develop decades after the initial infection. Interestingly, a 20-year prospective study of individuals performed in Colombia revealed that *H. pylori* eradication is crucial to reduce GC risk, together with endoscopic surveillance of gastric precancerous lesions[1]. For better GC prevention, the development of improved endoscopes is mandatory, since no macroscopic differences are observable between normal mucosa and pathological tissues at the stage of inflammatory and pre-neoplastic lesions, using classical and high-definition endoscopes. However, non-invasive real-time methods have been developed to detect mucosal inflammation related to *H. pylori* in both mice and humans; they are based on modifications of reemitted light at 560 nm, 600 nm and 640 nm, depending on the presence of gastric lesions[2].

In a search for virulence mechanisms that orchestrate alterations of gastric epithelial cells, two membrane proteins, namely Hp0305 and Hp1564, emerged that increase *H. pylori*-mediated inflammation and GC risk by promoting bacteria-gastric cell interactions that facilitate delivery of CagA into target cells[3]. The most commonly mutated gene in GC (40% cases) is the tumor suppressor gene p53, a key regulator of cell genomic stability. The fact that an *H. pylori* infection promotes p53 proteasomal degradation and inhibits the expression of USF1, the transcription factor known for its p53 stabilizing role in response to genotoxic stress, was previously established. Costa et al[4] showed the importance of studying *H. pylori* 7.13 strain in gastric epithelial cell lines where the bacterium delocalizes USF1 to foci close to the cell membranes and prevents USF1/p53 nuclear translocation, two events that are associated with the genotoxic activity of *H. pylori* infection[4].

Three interesting studies[5-7] pointed toward potential therapeutic approaches aimed at alleviating the *H. pylori*-induced pathogenesis. The first, focused on the membrane raft-mediated toxin function, showed that the *Campylobacter jejuni* cytolethal distending toxin C (CdtC) competes with the interactions between cholesterol-rich microdomains and bacterial actions, including VacA delivery, CagA translocation, and bacterial internalization in gastric epithelial cells[6]. The second demonstrated that sodium butyrate reduces *H. pylori*-induced inflammation by weakening the activity and production of virulence factors, thus providing new insight into the mechanisms by which diet-derived metabolites influence the development of *H. pylori*-induced disease and supporting the possibility of the butyrate supplementation as a strategy to relieve gastrointestinal symptoms in *H. pylori*-infected patients[7]. In the last, Gobert et al[5] demonstrated that dicarbonyl electrophiles, such as isolevuglandins, generated from lipid peroxidation during the inflammatory response, represent a link between the latter and somatic genomic alterations. The evidence that a potent scavenger of electrophiles, 5-ethyl-2-hydroxybenzylamine, reduces carcinogenesis in transgenic FVB/N insulin-gastrin (INS-GAS) mice and Mongolian gerbils' models of *H. pylori*-induced carcinogenesis supports the use of such scavengers as cancer chemopreventive agents.

Epigenetic alterations have been proposed as key initiating events in tumorigenesis occurring very early in cancer development and the capacity of *H. pylori* to induce DNA hypermethylation has been established[8]. Jan et al[9] found that IL-6, a cytokine promoting the proliferation and differentiation of various malignant tumor cells through the JAK/STAT3 pathway, is expressed in *H. pylori*-infected tumor tissues. The termination and modulation of the IL-6-JAK-STAT3 signaling pathway physiologically mediated by the suppressor of cytokine signaling (SOCS) inhibitors, is impaired in the gastric mucosa of GC patients, because *H. pylori* infection causes the inactivation of the SOCS1 gene through the hypermethylation of the promoter region. The consequent overactivation of the JAK/STAT3 signaling cascade results in an abnormal proliferation of gastric mucosal cells. Moreover, concerning *H. pylori*-induced DNA methylation, Takeshima et al[10] demonstrated that expression of TET genes, methylation erasers, is downregulated in inflamed mouse and human tissues; this is caused by the NF-κB -dependent upregulation of TET-targeting miRNAs, such as miR20A, miR26B, and miR29C. A vicious combination of TET repression and DNA methyltransferase activation, due to nitric

oxide production, is responsible for aberrant methylation induction in human gastric tissues. It has been shown that *H. pylori* infection, in gastric epithelial cells, induces phosphorylation of STAT3, on Tyr705[11] as well as on Ser727, and the resulting p-STAT3^{Ser727} is detected predominantly in mitochondria, together with LC3, the autophagosomal membrane-associated protein. This evidence supports the notion that p-STAT3^{Ser727} could be involved in clearance of damaged mitochondria by mitophagy. Although mitophagy can specifically eliminate dysfunctional mitochondria to maintain cellular homeostasis, overactivated mitophagy may aggravate the inflammation, provoking pathological conditions[12]. Interestingly, Courtois et al[13] demonstrated that a co-expression of the GC stem cell marker CD44 and the autophagic marker LC3, in mice and human stomach tissues infected with *H. pylori*, lead to autophagy GC stem cell emergence and could represent an interesting therapeutic target for GC[13]. A modulation of p-STAT3^{S727} was also reported by Xu et al[14], who demonstrated that in gastric epithelial cells, CagA enhances the recruitment of PKCδ via the eukaryotic translation elongation factor 1-alpha1 (eEF1A1), which subsequently increases the p-STAT3^{S727} content in the nucleus thus enhancing the expression of IL-6. The evidence that STAT3 is phosphorylated in Ser727 in gastric epithelial cells following *H. pylori* infection, has been questioned by Balic et al[15] who showed that, in contrast to p-STAT^{Tyr705} which is upregulated in *Helicobacter*-infected tissue, p-STAT3^{Ser727} is constitutively expressed in the gastric mucosa (mouse and human) irrespective of Helicobacter status. However, the same authors revealed that p-STAT3^{Ser727} is required for pre-neoplastic Helicobacter-induced gastritis, hyperplasia, and intestinal metaplasia lesions within the gastric compartment. Furthermore, Teng et al[16] showed that, in the gastric mucosa of patients and mice with *H. pylori* infection, the CagA-dependent tyrosine phosphorylation of STAT3 is paralleled by increased expression of the transcription factor BHLHE40, strongly upregulated in GC. The interaction between BHLHE40 and p-STAT3^{Tyr705} mediates the production of CXCL12 by the infected gastric epithelial cells resulting in the accumulation of CD4$^+$ T cells within the gastric mucosa. It has been also shown that CagA activates the mTOR Complex 1 (mTORC1) which, in turn, promotes the expression and release of proinflammatory cytokines, chemokines, and an antimicrobial peptide from gastric epithelial cells[17].

Complex Interactions Among *H. Pylori*, NOD1, NLP3, APRIL, BAFF and Gastric Lymphomagenesis

Different toll like receptors (TLRs) and cytokines are upregulated or downregulated during *H. pylori* infection. The nucleotide binding oligomerization domain 1 (NOD1) is an important sensor for *H. pylori* peptidoglycan, strongly dependent on the bacterial type IV "syringe" that is encoded by the *cag* pathogenicity island (*cag*PAI)[18]. The *cag*PAI, 2/NOD2, and NLP3 represent integrated check points which contribute to the regulation of IL-1β production.

H. pylori induces a strong inflammatory response that is directed at clearing the infection but, if it is not controlled, the response can be harmful to the host. *H. pylori* stimulates macrophages, both *in vitro* and *in vivo*, to produce the proliferation inducing ligand (APRIL), a crucial cytokine able to promote lymphomagenesis and B-cell proliferation and abundantly expressed in gastric MALT lymphoma (GML)[19]. Lymphoma-infiltrating macrophages are a major gastric source of APRIL. By using a lymphomagenesis model based on *Helicobacter* sp. infection of transgenic C57BL6 mice expressing the human form of the APRIL cytokine (Tg-hAPRIL), Blosse et al[20] characterized the gastric mucosal inflammatory response associated with GML and showed that all T cell subtypes infiltrate GML, including regulatory T cells, both in the animal model and in the human gastric microenvironment of GML patients. It is plausible that the regulatory T cell response might contribute to the persistence of the pathogen in the gastric mucosa by delaying the inflammatory response to allow chronic antigen stimulation necessary for lymphoid proliferation. According to a previous report[19], authors found APRIL significantly dysregulated in human GML and revealed that the cytokine is mainly expressed by eosinophils, suggesting the pro-tumorigenic potential of these cells. By using an antibody which recognizes the secreted and internalized form of APRIL, they also confirmed that the target cells of the cytokine are B cells[20]. Beside APRIL, the cytokine BAFF also contributes to the B-cell lymphomagenesis during chronic *H. pylori* infection[21]. Chonwerawong et al[22] revealed that *H. pylori* upreg-

ulates NLRC5 expression in macrophages and gastric tissues of mice and humans and that this expression correlates with gastritis severity. However, by taking advantage of NLRC5-deficient macrophages and knockout mice with non-functional *Nlrc5* within the myeloid cell lineage, the authors found that NLRC5 negatively modulates the production of proinflammatory cytokines, including BAFF, *in vitro* and protects against the formation of mucosal B-cell lymphoid tissue formation in response to chronic Helicobacter infection in mice[22].

There are several reports showing that *H. pylori* activates the NLRP3 inflammasome during mouse infection[23], while very few studies are available on the NLRP3 inflammasome activation in human cell systems during *H. pylori* infection. Choi et al[24] showed that the bacterium activates NLRP3 in THP-1 derived macrophages and that some chalcone derivatives block the activation. On the other hand, using the same cell line, Pachathundikandi et al[25] demonstrated that an exogenous second signal is needed to fully activate NLRP3 inflammasome formation during *H. pylori* infection. Therefore, the fact that *H. pylori* activates NLRP3 in human cells remains a controversial issue.

H. pylori Suppression and Evasion of Immune Responses

The lifelong persistence of *H. pylori* in the human stomach suggests that the host response fails to clear the infection. Different *H. pylori* factors, such as VacA, γ-glutamyl transpeptidase, and arginase, have defined immunosuppressive activity. In particular, VacA exerts immune suppression of specific responses by acting either on antigen-presenting cells or on T cells[26-28]. It has been recently shown that during *H. pylori* infection phagocytic cells promote high bacterial loads rather than contributing to bacterial clearance, and the available data suggest that *H. pylori* persistence can be explained in part by the failure of the bacterium to be killed by professional phagocytes. Studies[29,30] show that the bacterium interferes with the phagosome maturation process leading to phagosomes with an altered degradative capacity, and to megasomes wherein *H. pylori* resists killing. Moreover, macrophages infected with *H. pylori* strongly reduce the exposure of HLA-II molecules on the plasma membrane, compromising the bacterial antigen presentation toward Th lymphocytes[31]. This affects the possibility for effector T cells to recognize and activate the killing potential of macrophages which, in turn would become a survival niche for the bacterium. Codolo et al[32] deciphered the mechanism responsible for the decrease in HLA-II expression in macrophages. They demonstrated that *H. pylori* downmodulates the class II major histocompatibility complex transactivator (CIITA), the master regulator for the expression of HLA class II genes, by upregulating some miRNA targeting CIITA, namely let -7f-5p, let-7i-5p, miR-146b-5p, and -185-5p[32].

H. pylori evades the innate immune system by a variety of mechanisms. One of these mechanisms is avoidance of recognition by TLRs, by modulating surface molecules, including LPS and flagellin. However, Tegtmeyer et al[33] showed that the protein CagY, present at the tip of the type IV secretion system (T4SS)-pilus, can bind to and activate TLR5, which typically recognizes flagellin, through its repeat domains. Notably, TLR5 expression is significantly induced by *H. pylori* infection and coincides with the grade of inflammation and gastric malignant progression in patients. A study aimed to unravel the contributions of specific signaling pathways within human conventional type 2 dendritic cells (cDC2s) to the composition of secreted cytokines and chemokines in *H. pylori* infection. Neuper et al[34] demonstrated that T4SS plays only a minor role in *H. pylori*-induced activation of cDC2s. They showed that the bacterium drives the secretion of inflammatory mediators, including IL-12 and IL-18, *via* TLR4 signaling, while it attenuates the release of IL-1β and other inflammatory cytokines *via* TLR10. Finally, the TLR2 pathway significantly blocks the release of CXCL1 and CXCL8, while it promotes the secretion of TNFα and GM-CSF. The IRF4 also contributes to the regulation of *H. pylori* inflammation. By investigating the phenotype of mice in which *Irf4* was ablated specifically in cDC2s upon challenge with *H. pylori*, Zhang et al[35] demonstrated that IRF4$^{\Delta DC}$ mice have an enhanced Th1 response which is responsible for a better control of bacterial infection. Furthermore, Nagata et al[36] revealed that the cholesteryl glucosides that *H. pylori* produces following the extraction of cholesterol from the cell membranes, after the binding to C-type lectin receptors, exacerbate gastric inflammation by augmenting both Th1 and Th17 responses, which, however, do not contribute to *H. pylori* clearance.

H. Pylori May Exert Several Peculiar Effects on Macrophages

Hypusination is a polyamine-dependent post-translational modification of the translation factor EIF5A. Two enzymes, deoxyhypusine synthase (DHPS) and deoxyhypusine hydroxylase (DOHH) are responsible for hypusination. Gobert et al[37] found that *H. pylori* infection increases the levels of DHPS in murine macrophages, both *in vitro* and *in vivo*, resulting in the translation of multiple antimicrobial effectors. Consequently, mice with myeloid cell-specific deletion of *Dhps* exhibited an increased bacterial burden and inflammation *in vivo*[37].

The existence of a causal relationship between *H. pylori* infection and atherosclerosis is still a subject of debate, more than 25 years after the idea was first proposed[38]. Krupa et al[39] set up an *in vivo* model of *H. pylori* infection in guinea pigs exposed to a high fat diet, with the aim to investigate the role of the bacterium and dietary components, acting separate and in cooperation, in the development of a proatherogenic endothelial cell environment. The histological analysis showed that none of the animals from the *H. pylori*-infected groups develop typical atherosclerotic plaques in their aortic vessels, even in the presence of fatty substances. However, some leukocytes were shown to adhere to the aortic wall of animals infected with *H. pylori* and exposed to a high-fat diet, suggesting the generation of inflammatory conditions. Moreover, following an investigation on cells isolated from guinea pigs, the authors demonstrated that soluble *H. pylori* components induce transformation of macrophages into foam cells *in vitro* and influence the endothelial life span[39].

H. PYLORI AND ACQUIRED IMMUNITY

B cells and T helper cells exert specific host defense against *H. pylori via* different types of humoral and cellular pathways. However, it is well known that either B cells or T cells, instead of protecting the host, might contribute to different gastric immunopathologies and the development of severe forms of gastroduodenal diseases, such as GC, GML, peptic ulcer and gastric autoimmunity[40-45].

In the course of natural *H. pylori* infection, specific B cells are activated, and specific antibodies are produced. However, they are not able to clear the infection. The presence and the levels of *H. pylori* specific antibodies are very useful for diagnostic purposes. Alberts et al[46] demonstrated that *H. pylori* seroprevalence is high among first-generation migrants in the Netherlands and remains elevated among second-generation migrants. This study implies that individuals with a migrant background have a high exposure to *H. pylori*, especially to the more virulent *cag*A$^+$ strains, and highlights the need for a tailored prevention of gastric diseases among these individuals[46].

A very interesting study[47] identified a panel of putative specific antigens/epitopes for three different pathological outcomes of *H. pylori* infection, namely autoimmune gastritis (AIG), GC and GML. The novelty of this study is the approach, based on the use of the entire protein repertoire of the bacterium. A library of *H. pylori* protein fragments has been displayed on the surface of phages, and sera of infected individuals who developed GC, AIG, and GML were used as bait. Among a plethora of antigens identified, Soluri et al[47] validated protein CagY as a marker of *H. pylori* infection and progression towards gastric malignancies.

Another bioinformatic analysis was performed to investigate the impact of *H. pylori* on B cell function, with the aim of getting new insight into the mechanisms leading to GML formation[48]. By using a B cell line, Chichirau et al[48] were able to analyze the global transcriptome of infected cells by RNA sequencing and demonstrated that inflammation and migration-associated genes are downregulated, whereas central signal transduction regulators of cell survival and death are upregulated.

In a very elegant study, Ding et al[49] performed a transcriptome analysis for both coding genes and non-coding genes using flow-sorted myeloid-derived suppressor cells (Schlafen4$^+$ MDSCs) from the stomach of Helicobacter-infected mice, to define the underlying mechanism that drives the emergence of this specific subset of MDSCs in the stomach during Helicobacter-induced metaplasia, and to investigate its role in tumor progression[49]. The study revealed that miR130b is induced in Schlafen4$^+$ MDSCs is required for acquisition of the suppressive effect on T cells and promotes Helicobacter-induced metaplasia. Interestingly, high levels of miR130b were found in patients' sera with atrophy, and GC[49].

In *H. pylori* infection, activation of both Th1 and Th17 cells occurs *in vivo* with subsequent production of IFN-γ, IL-17, and TNF–α[40,43-45].

New data highlighted that in HIV-infected adults, gut-homing Th1 also plays a key role in the development of chronic gastritis, peripheral blood gut-homing $CD4^+$ T cells being significantly higher in individuals with histological gastritis compared with those without chronic gastritis[50]. Chen et al[51] demonstrated that CD103, a member of the integrin family, expressed by immune cells and contributing to their tissue-specific localization, is upregulated in gastric $CD4^+$ T cells from *H. pylori*-positive patients. Authors showed that CD103 is associated with the differentiation, proliferation and cytokine production of gastric $CD4^+$ T cells. More importantly, CD103 acts as a costimulatory molecule. By interacting with TCR, it enhances CD3ζ/ZAP70 signaling, which are both essential for proliferation and pro-inflammatory cytokine production by gastric $CD4^+$ T cells[51].

Furthermore, concerning costimulatory molecules expressed by cells accumulating in the gastric mucosa of *H. pylori*-infected patients, Ming et al[52] observed the up-regulation of OX40 in mucosal-associated invariant T (MAIT) cells, a class of innate-like T cells that recognize the small-molecule derivatives produced by microbes during riboflavin synthesis. Authors demonstrated that OX40 promotes IL-9 production by MAIT cells in *H. pylori*-positive gastritis patients and that the cytokine level correlates with mucosal inflammation[53].

H pylori has been reported to increase the expression of ACE-2 receptors in the gastrointestinal tract[54]. Accordingly, Balamtekin et al[55] reported that *H. pylori* increases diarrhea and abdominal pain in COVID-19 patients, probably because it favors the entry of the virus into enterocytes. This conclusion is corroborated by another study showing that the stomach with a *H. pylori* infection history and intestinal metaplasia might represent a susceptibility factor to SARS-CoV-2 infection[56].

VACCINES

As in any severe infectious disease, a vaccine to prevent or a strategy to treat *H. pylori* infection is needed. In the past, most vaccine studies preferentially used urease, the cytotoxin VacA, CagA, or HP-NAP as antigens of choice. Investigators continue to evaluate potential new antigens and novel approaches for production and delivery of vaccine antigens. There are three key requirements for developing an effective vaccine against *H. pylori*: appropriate bacterial antigens, safe and effective adjuvants, and use of an appropriate route of delivery. However, as we know, last year all forces around the world were devoted to the design and production of a vaccine against SARS-CoV-2.

To set-up a novel vaccine against SARS-CoV-2, *H. pylori* has proved very useful. *H. pylori* nonheme ferritin, which self assembles in nanoparticles, has been produced as a chimera with the influenza antigen HA, and showed a major increment in influenza protection in animal models. It elicited a robust humoral immune response against a broad spectrum of H1N1 viruses[57]. One influenza HA *H. pylori* ferritin vaccine has completed phase I clinical trial (NCT03186781). Another *H. pylori* ferritin-based influenza H1 vaccine is currently in phase I (NCT03814720). Ma et al[58] chose *H. pylori* ferritin conjugated with the receptor binding domain (RBD) or with heptad repeat (HR) of SARS-CoV-2 to obtain self-assembling nanoparticles. This formulation elicited neutralizing antibodies in rhesus macaques, as well as T and B cell responses prior to boost immunization. The responses persisted for more than three months[58]. Kim et al[59] reported the development of *H. pylori* ferritin nanoparticles in which ferritin was expressed as a chimera with the RBD. RBD-ferritin protein was purified from mammalian cells and administered to ferrets by intramuscular or intranasal inoculation. The latter produced potent neutralizing antibodies against SARS-CoV-2. Vaccinated ferrets were efficiently protected from the SARS-CoV-2 challenge, showing no fever, body weight loss, or clinical symptoms. Furthermore, vaccinated ferrets showed rapid clearance of infectious virus in nasal washes and lungs as well as viral RNA in respiratory organs. RBD- and HR-based nanoparticles thus represent a promising vaccination approach against SARS-CoV-2 and other coronaviruses.

The appeal of nanoparticle immunogens lies in their inherent multivalent display of antigens, which is known to elicit robust B cell responses. Another important factor to consider in viral immunogen design is glycosylation. Zhang et al[60] developed a modular self-assembling

nanoparticle platform that allows for the plug-and-play display of trimeric viral glycoproteins on nanoparticle surfaces, again taking advantage of the *H. pylori* ferritin or, as an alternative, *Aquifex aeolicus* lumazine synthase (Lus). The multivalent presentation of the SARS-CoV-2 spike on nanoparticles was shown to elicit substantially higher neutralizing responses than the spike.

It is well known that *H. pylori* is inversely associated with allergic diseases. The main reason for this is that HP-NAP can elicit Th1 immune responses that downmodulate the Th2 inflammation that represents the immunopathological basis of allergic inflammation[61]. Zhang et al[62] engineered a *Lactococcus lactis* which expresses HP-NAP and evaluated its effects on food allergy in Balb/c mice. They showed that it improves food allergy symptoms (acute diarrhea and intestinal inflammation) and decreases serum histamine levels. In addition, the secretion of OVA-specific IgG2a, IFN-γ was promoted and the level of IL-4 and IgE was restrained, suggesting that the recombinant bacterium might be a promising approach for prevention and treatment of food allergy.

In an interesting study, Rhaman et al[63] took advantage of computational biology for therapeutic drug target identification and a multi-epitope vaccine against multi-strains of *H. pylori*. They started from the core proteome of 84 *H. pylori* strains that were analyzed for comparative sequences and different biological updated databases to prioritize druggable proteins. Vaccine constructs were designed, based on overlapped MHC-I, MHC-II and B cell epitopes. One vaccine construct was identified as the least allergenic and most antigenic and as a promiscuous vaccine to elicit host immune response.

CONCLUSIONS

H. pylori induces innate and acquired responses in the stomach, with activation of neutrophils, macrophages, eosinophils, T and B cells. Both TLR and NOD take part in host innate defense in *H. pylori*. Inflammatory Th1/Th17 responses occur in the stomach of infected patients. Different factors, related to genetics, age, sex, other infections, diet, and environment, influence the individual host gastric immune responses. Different Helicobacter factors, such as VacA, CagA, and HP-NAP, are able to elicit either harmful or protective immune responses. The infected patients usually fail to clear the infection, although vigorous immune responses are apparently mounted. Altogether the investigations performed so far highlight the crucial roles played by both *H. pylori* and the immunopathological responses of the host in the pathogenesis of GC, GML, and gastric autoimmunity, suggesting the strong and urgent need for an anti-*H. pylori* vaccine in order to prevent both the infection and the severe diseases due to *H. pylori*.

Acknowledgements

We thank the Italian Ministry of University and Research, the Italian Ministry of Industry, the Italian Ministry of Health, and the Associazione Italiana per la Ricerca sul Cancro for their support of our studies.

Conflict of interest

The authors have no competing interests.

REFERENCES

1. Piazuelo MB, Bravo LE, Mera RM, Camargo MC, Bravo JC, Delgado AG, Washington MK, Rosero A, Garcia LS, Realpe JL, Cifuentes SP, Morgan DR, Peek RM Jr, Correa P, Wilson KT. The Colombian chemoprevention trial: 20-year follow-up of a cohort of patients with gastric precancerous lesions. Gastroenterology 2021; 160: 1106-1117.
2. Bazin T, Martinez-Herrera SE, Jobart-Malfait A, Benezeth Y, Boffety M, Julié C, Emile JF, Michel V, Goudail F, Touati E, Marzani F, Lamarque D. Multispectral imaging detects gastritis consistently in mouse model and in humans. Sci Rep 2020; 10: 20047.
3. Varga MG, Wood CR, Butt J, Ryan ME, You WC, Pan K, Waterboer T, Epplein M, Shaffer CL. Immunostimulatory membrane proteins potentiate H. pylori-induced carcinogenesis by enabling CagA translocation. Gut Microbes 2021; 13: 1-13.
4. Costa L, Corre S, Michel V, Le Luel K, Fernandes J, Ziveri J, Jouvion G, Danckaert A, Mouchet N, Da Silva Barreira D, Torres J, Camorlinga M, D'Elios MM, Fiette L, De Reuse H, Galibert MD, Touati E. USF1 defect drives p53 degradation during *Helicobacter pylori* infection and accelerates gastric carcinogenesis. Gut 2020; 69: 1582-1591.

5. Gobert AP, Boutaud O, Asim M, Zagol-Ikapitte IA, Delgado AG, Latour YL, Finley JL, Singh K, Verriere TG, Allaman MM, Barry DP, McNamara KM, Sierra JC, Amarnath V, Tantawy MN, Bimczok D, Piazuelo MB, Washington MK, Zhao S, Coburn LA, Wilson KT. Dicarbonyl electrophiles mediate inflammation-induced gastrointestinal carcinogenesis. Gastroenterology 2021; 160: 1256-1268.
6. Yeh JY, Lin HJ, Kuo CJ, Feng CL, Chou CH, Lin CD, Wu HY, Li CY, Chiu CH, Lai F. Campylobacter jejuni Cytolethal Distending Toxin C Exploits Lipid Rafts to Mitigate *Helicobacter pylori*-Induced Pathogenesis. Front Cell Dev Biol 2021; 8: 617419.
7. Huang Y, Ding Y, Xu H, Shen C, Chen X, Li C. Effects of sodium butyrate supplementation on inflammation, gut microbiota, and short-chain fatty acids in *Helicobacter pylori*-infected mice. Helicobacter 2021; 26: e12785.
8. Touati E. When bacteria become mutagenic and carcinogenic: lessons from H. pylori. Mutat Res 2010; 703: 66-70.
9. Jan I, Rather RA, Mushtaq I, Malik AA, Besina S, Baba AB, Farooq M, Yousuf T, Rah B, Afroze D. *Helicobacter pylori* subdues cytokine signaling to alter mucosal inflammation via hypermethylation of suppressor of cytokine signaling 1 gene during gastric carcinogenesis. Front Oncol 2021; 10: 604747
10. Takeshima H, Niwa T, Yamashita S, Takamura-Enya T, Iida N, Wakabayashi M, Nanjo S, Abe M, Sugiyama T, Kim YJ, Ushijima T. TET repression and increased DNMT activity synergistically induce aberrant DNA methylation. J Clin Invest 2020; 130: 5370-5379.
11. Bronte-Tinkew DM, Terebiznik M, Franco A, Ang M, Ahn D, Mimuro H, Sasakawa C, Ropeleski MJ, Peek RM Jr, Jones NL. *Helicobacter pylori* cytotoxin-associated gene A activates the signal transducer and activator of transcription 3 pathway in vitro and in vivo. Cancer Res 2009; 69: 632-639.
12. Piao JY, Kim SJ, Kim DH, Park JH, Park SA, Han HJ, Na HK, Yoon K, Lee HN, Kim N, Hahm KB, Young-Joon Surh YJ. *Helicobacter pylori* infection induces STAT3 phosphorylation on Ser727 and autophagy in human gastric epithelial cells and mouse stomach. Sci Rep 2020; 10:15711.
13. Courtois S, Haykal M, Bodineau C, Sifré E, Lamia Azzi-Martin L, Ménard A, Mégraud F, Lehours P, Durán RV, Varon C, Bessède E. Autophagy induced by *Helicobacter pylori* infection is necessary for gastric cancer stem cell emergence. Gastric Cancer 2021; 24: 133-144.
14. Xu S, Wu X, Zhang X, Chen C, Chen H, She F. CagA orchestrates eEF1A1 and PKCδto induce interleukin-6 expression in *Helicobacter pylori*-infected gastric epithelial cells. Gut Pathog 2020; 12: 31.
15. Balic JJ, Saad MI, Dawson R, West AJ, McLeod L, West AC, D'Costa K, Deswaerte V, Dev A, Sievert W, Gough DJ, Bhathal PS, Richard L Ferrero, Jenkins BJ. Constitutive STAT3 serine phosphorylation promotes Helicobacter-mediated gastric disease. Am J Pathol 2020; 190: 1256-1270.
16. Teng YS, Zhao YL, Liu YG, Cheng P, Lv YP, Mao FY, Chen W, Yang SM, Hao CJ, Peng LS, Zhang JY, Zhang WJ, Zou QM, Zhuang Y. Up-expression of BHLHE40 in gastric epithelial cells increases CXCL12 production through interaction with p-STAT3 in *Helicobacter pylori*-associated gastritis. FASEB J 2020; 34: 1169-1181.
17. Feng GJ, Chen Y, Li K. *Helicobacter pylori* promote inflammation and host defense through the cagA-dependent activation of mTORC1. J Cell Physiol 2020; 235:10094-10108.
18. Chonwerawong M, Ferrero RL. Analysis of innate immune responses to *Helicobacter pylori*. Methods Mol Biol 2021; 2283: 191-214.
19. Munari F, Lonardi S, Cassatella MA, Doglioni CA, Cangi MG, Amedei A, Facchetti F, Eishi Y, Rugge M, Fassan M, de Bernard M, D'Elios MM, Vermi W. Tumor-associated macrophages as major source of APRIL in gastric MALT lymphoma. Blood 2011; 117: 6612-6616.
20. Blosse A, Peru S, Levy M, Marteyn B, Floch P, Sifré E, Giese A, Prochazkova-Carlotti M, Azzi Martin L, Dubus P, Mégraud F, Ruskone Fournestraux A, Fabiani B, Copie Bergman C, Robe C, Hahne M, Huard B, Lehours P. APRIL-producing eosinophils are involved in gastric MALT lymphomagenesis induced by Helicobacter sp infection. Sci Rep 2020; 10: 14858.
21. Munari F, Fassan M, Capitani N, Codolo G, Villa-Caballer M, Pizzi M, Rugge M, Della Bella C, Troilo A, D'Elios S, Baldari CT, D'Elios MM, de Bernard M. Cytokine BAFF released by *Helicobacter pylori*-infected macrophages triggers the th17 response in human chronic gastritis. J Immunol 2014; 193: 5584-5594.
22. Chonwerawong M, Ferrand M, Chaudry HM, Higgins C, Tran LS, Lim SS, Walker MM, Bhathal PS, Dev A, Moore GT, Sievert W, Jenkins BJ, D'Elios MM, Philpott DJ, Kufer TA, Ferrero RL. Innate immune molecule NLRC5 protects mice from Helicobacter-induced formation of gastric lymphoid tissue. Gastroenterology 2020; 159: 169-182.
23. Pachathundikandi SK, Müller A, Backert S. Inflammasome activation by *Helicobacter pylori* and Its Implications for Persistence and Immunity. Curr Top Microbiol Immunol 2016; 397: 117-131.
24. Choi HR, Lim H, Lee JH, Park H, Kim HP. Interruption of *Helicobacter pylori*-Induced NLRP3 inflammasome activation by chalcone derivatives. Biomol Ther (Seoul) 2021. doi: 10.4062/biomolther.2020.192. Online ahead of print.
25. Pachathundikandi SK, Blaser N, Bruns H, Backert S. *Helicobacter pylori* Avoids the Critical Activation of NLRP3 Inflammasome-Mediated Production of Oncogenic Mature IL-1β in Human Immune Cells. Cancers (Basel) 2020; 12: 803.
26. Molinari M, Salio M, Galli C, Norais N, Rappuoli R, Lanzavecchia A, Montecucco C. Selective inhibition of Ii-dependent antigen presentation by *Helicobacter pylori* toxin VacA. J Exp Med 1998; 187: 135-140.
27. Boncristiano M, Rossi Paccani S, Barone S, Ulivieri C, Patrussi L, Ilver D, Amedei A, D'Elios MM, Telford JL, Baldari CT. The *Helicobacter pylori* vacuolating toxin inhibits T cell activation by two independent mechanisms. J Exp Med 2003; 198: 1887-1897.
28. Utsch C, Haas R. VacA's induction of VacA-containing vacuoles (VCVs) and their immunomodulatory activities on human T cells. Toxins (Basel) 2016; 190.
29. Tegtmeyer N, Backert S. Different roles of integrin-beta1 and integrin-alphav for type IV secretion of CagA versus cell elongation phenotype and cell lifting by *Helicobacter pylori*. PLoS Pathog 2020; 16: e1008135.

30. Allen LA, Schlesinger LS, Kang B. Virulent strains of *Helicobacter pylori* demonstrate delayed phagocytosis and stimulate homotypic phagosome fusion in macrophages. J Exp Med 2000; 191: 115-128.
31. Pagliari M, Munari F, Toffoletto M, Lonardi S, Chemello F, Codolo G, Millino C, Della Bella C, Pacchioni B, Vermi W, Fassan M, de Bernard M, Cagnin S. *Helicobacter pylori* affects the antigen presentation activity of macrophages modulating the expression of the immune receptor CD300E through miR-4270. Front Immunol 2017; 8: 1288.
32. 32.Codolo G, Toffoletto M, Chemello F, Coletta S, Soler Teixidor G, Battaggia G, Munari G, Fassan M, Cagnin S, de Bernard M. *Helicobacter pylori* dampens HLA-II expression on macrophages via the up-regulation of miRNAs targeting CIITA. Front. Immunol 2020; 10: 2923.
33. Tegtmeyer N, Neddermann M, Lind J, Pachathundikandi JK, Sharafutdinov I, Gutiérrez-Escobar AJ, Brönstrup M, Tegge W, Hong M, Rohde M, Delahay RM, Vieth M, Sticht H, Backert S. Toll-like receptor 5 activation by the CagY repeat domains of *Helicobacter pylori* Cell Rep 2020; 32: 108159.
34. Neuper T, Frauenlob T, Sarajlic M, Posselt G, Wessler S, Horejs-Hoeck J. TLR2, TLR4 and TLR10 shape the cytokine and chemokine release of H. pylori-infected human DCs. Int J Mol Sci 2020; 21: 3897.
35. Zhang X, Artola-Boran M, Fallegger A, Arnold IC, Weber A, Reuter S, Taube C, Müller A. IRF4 expression is required for the immunoregulatory activity of conventional type 2 dendritic cells in settings of chronic bacterial infection and cancer. J Immunol 2020; 205: 1933-1943.
36. Nagata M, Toyonaga K, Ishikawa E, Haji S, Okahashi N, Takahashi M, Izumi Y, Imamura A, Takato K, Ishida H, Nagai S, Illarionov P, Stocker BL, Timmer MSM, Smith DGM, Williams SJ, Bamba T, Miyamoto T, Arita, Appelmelk BJ, Yamasaki S. *Helicobacter pylori* metabolites exacerbate gastritis through C-type lectin receptors. J Exp Med 2021; 218: e20200815.
37. Gobert AP, Finley JL, Latour YL, Asim M, Smith TM, Verriere TG, Barry DP, Allaman MM, Delagado AG, Rose KL, Calcutt MW, Schey KW, Sierra JC, Piazuelo MB, Mirmira RG, Wilson KT. Hypusination orchestrates the antimicrobial response of macrophages. Cell Rep 2020; 33:108510.
38. Mendall, MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, Camm AJ, Northfield TC. Relation of *Helicobacter pylori* infection and coronary heart disease. Heart 1994; 71: 437-439.
39. Krupa A, Gonciarz W, Rusek-Wala P, Rechciński T, Gajewski A, Samsel Z, Dziuba A, Śmiech A, Chmiela M. *Helicobacter pylori* infection acts synergistically with a high-fat diet in the development of a proinflammatory and potentially proatherogenic endothelial cell environment in an experimental model. Int J Mol Sci 2021; 22: 3394.
40. D'Elios MM, Manghetti M, De Carli M, Costa F, Baldari CT, Burroni D, Telford JL, Romagnani S, Del Prete G. T helper 1 effector cells specific for *Helicobacter pylori* in the gastric antrum of patients with peptic ulcer disease. J Immunol 1997; 158: 962-967.
41. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991; 338: 1175-1176.
42. D'Elios MM, Amedei A, Manghetti M, Costa F, Baldari CT, Quazi AS, Telford JL, Romagnani S, Del Prete G. Impaired T-cell regulation of B-cell growth in *Helicobacter pylori*-related gastric low grade MALT lymphoma. Gastroenterology 1999; 117: 1105-1112.
43. D'Elios MM, Appelmelk BJ. Amedei A, Bergman MP, Del Prete G. Gastric autoimmunity: the role of *Helicobacter pylori* and molecular mimicry. Trends Mol Med 2004; 10: 316-323.
44. Amedei A, Munari F, Della Bella C, Niccolai E, Benagiano M, Bencini L, Cianchi F, Farsi M, Emmi G, Zanotti G, de Bernard M, Kundu M, D'Elios MM. *Helicobacter pylori* secreted peptidyl prolyl cis, trans-isomerase drives Th17 inflammation in gastric adenocarcinoma. Intern Emerg Med 2014; 9: 303-309.
45. Capitani N, Codolo G, Vallese F, Minervini G, Grassi A, Cianchi F, Troilo A, Fischer W, Zanotti G, Baldari CT, de Bernard M, D'Elios MM. The lipoprotein HP1454 of *Helicobacter pylori* regulates T-cell response by shaping T-cell receptor signalling. Cell Microbiol 2019; 21: e13006.
46. Alberts CJ, Jeske R, de Martel C, den Hollander WJ, Michel A, Prins M, Snijder MB, van der Loeff MF, Waterboer T. *Helicobacter pylori* seroprevalence in six different ethnic groups living in Amsterdam: the HELIUS study. Helicobacter 2020; 25: e12687.
47. Soluri MF, Puccio S, Caredda G, Edomi P, D'Elios MM, Cianchi F, Troilo A, Santoro C, Sblattero D, Peano C. Defining the *Helicobacter pylori* disease specific antigenic repertoire. Front.Microbiol 2020; 11: 1551.
48. Chichirau BE, Scheidt T, Diechler S, Neuper T, Horejs-Hoeck J, Huber CJ, Posselt G, Wessler S. Dissecting the *Helicobacter pylori*-regulated transcriptome of B cells. Pathog Dis 2020;78: ftaa049.
49. Ding L, Li Q, Chakrabarti J, Munoz A, Faure-Kumar E, Ocadiz-Ruiz R, Razumilava N, Zhang G, Hayes MH, Sontz RA, Mendoza ZE, Mahurkar S, Greenson JK, Perez-Perez G, Hanh NTH, Zavros Y, Samuelson LC, Iliopoulos D, Merchant JL. MiR130b from Schlafen4 + MDSCs stimulates epithelial proliferation and correlates with preneoplastic changes prior to gastric cancer. Gut 2020; 9: 1750-1761.
50. Sato H, Ota Y, Kido Y, Matsumoto T, Matsubara Y, Matano T, Hirata Y, Kawana-Tachikawa A, Yamaoka Y, Yotsuyanagi H, Adachi E. Gut-goming CD4+ T Cells are associated with the activity of gastritis in HIV-infected adults. AIDS Res Hum Retroviruses 2020; 36: 910-917.
51. Chen P, Ming S, Lao J, Li C, Wang H, Xiong L, Zhang S, Liang Z, Niu X, Deng S, Geng L, Wu M, Wu Y, Gong S. CD103 promotes the pro-inflammatory response of gastric resident CD4 + T cell in *Helicobacter pylori*-positive gastritis. Front Cell Infect Microbiol 2020; 10: 436.
52. Hinks TSC, Zhang XW. MAIT cell activation and functions. Front Immunol 2020; 11: 1014.
53. Ming S, Zhang M, Liang Z, Li C, He J, Chen P, Zhang S, Niu X, Deng S, Geng L, Zhang G, Gong S, Wu Y. OX40L/OX40 signal promotes IL-9 production by mucosal MAIT cells during *Helicobacter pylori* infection. Front Immunol 2021; 12: 626017.
54. Gkogkou E, Barnasas G, Vougas K, Trougakos IP. Expression profiling meta-analysis of ACE2 and TMPRSS2, the putative anti-inflammatory receptor and priming protease of SARS-CoV-2 in human cells, and identification of putative modulators. Redox Biology 2020; 101615.

55. Balamtekin N, Artuk C, Arslan M, Gülœn M. The effect of *Helicobacter pylori* on the presentation and clinical course of Coronavirus disease 2019 infection. J Pediatr Gastroenterol Nutr 2021; 72: 511-513.
56. Zhang M, Feng C, Zhang X, Hu S, Zhang Y, Min M, Liu B, Ying X, Liu Y. Susceptibility factors of stomach for SARS-CoV-2 and treatment implication of mucosal protective agent in COVID-19. Front Med (Lausanne) 2021; 7: 597967.
57. Kanekiyo M, Wei CJ, Yasine HM, Mc Tanney PM, Boyington JC, Whittle JRR, Rao SS, Kong WP, Wang L, Nabel GJ. Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. Nature 2013; 499: 102-106.
58. Ma X, Zou F, Yu F, Li R, Yuan Y, Zhang Y, Zhang LX, Deng J, Chen T, Song Z, Qiao Y, Zhan Y, Liu J, Zhang J, Zhang X, Peng Z, Li Y, Lin Y, Liang L, Wang G, Chen Y, Chen Q, Pan T, He X, Zhang H. Nanoparticle vaccines based on the receptor binding domain (RBD) and heptad repeat (HR) of SARS-CoV-2 elicit robust protective immune responses immunity. J Immunol 2020; 53: 1315-1330.
59. Kim YI, Kim D, Yu KM, Seo HD, Lee SA, Casel MAB, Jang SG, Kim S, Jung WR, Lai CJ, Choi YK, Jung JU. Development of spike receptor-binding domain nanoparticles as a vaccine candidate against SARS-CoV-2 infection in ferrets. mBio 2021; 12: e00230-21.
60. Zhang B, Chao CW, Tsybovsky Y, Abiona OM, Hutchinson GB, Moliva JI, Olia AS, Pegu A, Phung E, Stewart-Jones GBE, Verardi R, Wang L, Wang S, Werner A, Yang ES, Yap C, Zhou T, Mascola JR, Sullivan NJ, Graham BS, Corbett KS, Kwong PD. A platform incorporating trimeric antigens into self-assembling nanoparticles reveals SARS-CoV-2-spike nanoparticles to elicit substantially higher neutralizing responses than spike alone. Sci Rep 2020; 10: 18149.
61. Codolo G, Mazzi P, Amedei A, Del Prete G, Berton G, D'Elios MM, de Bernard M. The neutrophil-activating protein of *Helicobacter pylori* down-modulates Th2 inflammation in ovalbumin-induced allergic asthma. Cell Microbiol 2008; 10: 2355-2363.
62. Zhang K, Mirza WA, Ni P, Yu M, Wang C, Wang B, Chang S, Yue L, Zhang R, Duan G. Recombination Lactococcus lactis expressing *Helicobacter pylori* neutrophil-activating protein A attenuates food allergy symptoms in mice. FEMS Microbiology Letters 2021; 368: fnab034.
63. Rahman N, Ajmal A, Ali F, Rastrelli L. Core proteome mediated therapeutic target mining and multi-epitope vaccine design for *Helicobacter pylori*. Genomics 2020; 112: 3473-3483.