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Helicobacter: Inflammation, immunology, and vaccines

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Abstract

Helicobacter pylori infection induces a chronic gastric inflammation which can lead to gastric ulcers and cancer. The mucosal immune response to *H. pylori* is first initiated by the activation of gastric epithelial cells that respond to numerous bacterial factors, such as the cytotoxin-associated gene A or the lipopolysaccharide intermediate heptose-1,7-bisphosphate. The response of these cells is orchestrated by different receptors including the intracellular nucleotide-binding oligomerization domain-containing protein 1 or the extracellular epidermal growth factor receptor. This nonspecific response leads to recruitment and activation of various myeloid (macrophages and dendritic cells) and T cells (T helper-17 and mucosal-associated invariant T cells), which magnify and maintain inflammation. In this review, we summarize the major advances made in the past year regarding the induction, the regulation, and the role of the innate and adaptive immune responses to *H. pylori* infection. We also recapitulate efforts that have been made to develop efficient vaccine strategies.

Keywords

host-pathogen interaction; mucosal immunity; stomach; virulence factors

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DISCLOSURES OF INTERESTS

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1 | INFLAMMATION

1.1 | *Helicobacter pylori* factors that regulate the activation of epithelial cells

Helicobacter pylori is essentially an extracellular bacterium that generally does not invade the gastric mucosa of the host. Gastric epithelial cells (GECs) are a primary target for *H. pylori* infection and actively contribute to the development of an acute and chronic inflammation. Natural immune response strongly depends on host recognition of invariant structures, namely pathogen-associated molecular patterns (PAMPs), via signaling through pattern recognition receptors such as toll-like receptors (TLRs). But *H. pylori* also regulates the host innate responses by injecting one of its main virulence factors, the cytotoxin-associated gene A (CagA), directly into epithelial cells using a type 4 secretion system (T4SS).

Thus, Li et al investigated the effect of *H. pylori* on GEC auto-phagy. Using immunohistochemistry, they demonstrated that the SQSTM1/p62 (sequestosome1) protein, which accumulates when autophagy is dysfunctional, accumulates in the gastric epithelium of patients infected with *cag*A^{+}/*vac*As1m2 *H. pylori* strains compared to uninfected individuals or to patients infected with *cag*A^{-}/*vac*As1m2 isolates.[1] Then, in vitro investigations demonstrated that the CagA dampens the formation of autophagy by stimulating the c-Met/Akt signaling pathway.[1] Furthermore, because the inhibition of autophagy by CagA was associated with an increase of proinflamma-tory cytokine release,[1] Li et al concluded that this mechanism is an unrecognized cellular event that may sustain gastric inflammation and consequently lead to gastric carcinogenesis.

Members of the Pellino proteins (PELI1, PELI2, PELI3) have been shown to sustain TLR signaling by functioning as scaffold proteins[2] and by inducing polyubiquitination of interleukin (IL)-1 receptor-associated kinase 1 (IRAK1).[3] TLR2 is involved in the recognition of *H. pylori* lipopolysaccharide (LPS) in GECs[4] and leads to the activation of numerous signaling events, to nuclear factor-kappa B (NF-κB) activation, and to the transcription of the genes encoding chemokines such as C-X-C motif chemokine ligand 8 (CXCL8, also known as IL-8), which plays a major function in the development of gastritis and gastric cancer.[5] Using TLR2-expressing HEK293T cells and *H. pylori* LPS stimulation, Smith et al demonstrated that PELI1 and PELI3 overexpression enhances NF-κB activation and *CXCL8* gene expression;[6] in contrast, the innate response was dampened by PELI3.[6] Moreover, *PELI1* mRNA expression was stimulated in the human GEC line MKN45 in response to *H. pylori* LPS and live bacteria.[6] This emphasizes that the balance between PELI1/PELI2 and PELI3 in *H. pylori*-infected patients might have effects on the development of gastritis. In the same way, in the last year, three groups reported the critical effect of an LPS intermediate metabolite, namely heptose-1,7-bisphosphate (HBP), on the innate response of GECs.[7–9] Using direct delivery of HBP in host cells and mutants defective in the genes required for synthesis of HBP or for the T4SS, they all found that HBP (a) is delivered intracellularly by a functional T4SS, (b) is sensed by tumor necrosis factor (TNF) receptor-associated factor-interacting protein with a forkhead-associated domain (TIFA), and (c) signals in GECs to stimulate an NF-κB-dependent innate immune response,[7–9] independently of nucleotide-binding oligomerization domain-containing

protein 1 (NOD1).[8] Further, Zimmermann et al proposed that TIFA, TNF receptor-associated factor 2 (TRAF2), and tumor growth factor-beta-activated kinase 1 binding protein 2 (TAB 2) form large protein complexes called TIFAsomes.[9] These discoveries have revived the lost interest in the role of *H. pylori* LPS on the induction of the immune response and the increased complexity of the interaction between the T4SS and GECs. Now, it is important to understand whether the *H. pylori* HBP/TIFA signaling pathway also has a biological significance in myeloid cells.

In an intriguing manner, in this last year, it has been reported that *H. pylori* also induces a rapid noncanonical NF-κB signaling in human HeLa cells and in the GEC line NCI-N87, characterized by an increase in the expression of the mitogen-activated protein kinase kinase kinase 14 (MAP3K14, also named NIK) and by phosphorylation/activation of p100.[10] The authors then questioned the cellular mechanism leading to the activation of this pathway. The phosphorylation of p100 was not observed when a *virB7* mutant strain, which lacks a functional T4SS, was used to stimulate GECs; in contrast, a *cag*A-deficient strain had the same effect as the parental strain,[10] demonstrating that the T4SS, but not CagA, is required for this signaling event. The authors also demonstrated that MAP3K14 induction and p100 activation are dependent on the lymphotoxin beta receptor that signals via TRAF2.[10] At last, they found that MAP3K14 protein expression is also increased in gastric biopsies from *H. pylori*-infected patients compared to uninfected individuals,[10] providing a further rationale that NIK could represent a marker of *H. pylori* infection and may have a potential effect in *H. pylori*-mediated diseases. In the light of the finding that HBP,[7–9] peptidoglycan,[11] and DNA[12] can be transferred into GECs by a T4SS, it would be of interest to understand how *H. pylori* signals in cells activate the noncanonical NF-κB pathway.

Helicobacter pylori, which is an auxotroph for cholesterol, extracts the lipid from host membranes to incorporate it into its own outer membrane using the enzyme cholesterol-α-glucosyltransferase (Cgt).[13] Cholesterol glucosylation and extraction from host cells result in lipid raft destruction and/or alteration of the membrane architecture, which has been linked to immune evasion and bacterial persistence.[13] Phosphorylation/activation of signal transducer and activator of transcription 1 (STAT1) is stimulated in the GEC lines MKN45 and AGS, as well as in primary human GEC, treated with interferon-gamma (IFN-γ), but not in these cells preinfected for 24 h with *H. pylori*.[14] This phenomenon was not observed with an *H. pylori* mutant strain lacking *cgt*. Furthermore, *H. pylori* coated with water-soluble cholesterol before infection did not block the cellular response to IFN-γ.[14] At last, the reduction of cholesterol levels by *H. pylori* in GEC blocks IFN-γ signaling by preventing assembly of IFN-γ receptor subunits via lipid raft destruction.[14] In an important way, IL-6 and IL-22 signaling in GEC was also perturbed by *H. pylori* via a mechanism involving a Cgt-dependent cholesterol depletion. In mice infected for 3 days, the *H. pylori* PMSS1 Δ*cgt* was not able to colonize mice as the wild-type strain did but the expression of the host IFN-γ downstream response gene *Irf1* was significantly higher in PMSS1 Δ*cgt*-infected mice compared to animals infected with the parental strain.[14] Therefore, *H. pylori* Cgt reduces cholesterol levels in infected GEC, thereby blocking T-cell signaling, allowing the bacteria to escape the host inflammatory response.

1.2 | Regulation of inflammation by cellular receptors

Intracellular and extracellular receptors of PAMPS or immune effectors display different effects on *H. pylori* pathogenesis. NOD1 is expressed in GECs, senses peptidoglycan, and modulates innate immune response.[12] Tran et al investigated whether NOD1 can also dampen the inflammatory response by inducing the cytokine IL-33. Indeed, the mouse GEC line GSM06 infected with *H. pylori* releases the anti-inflammatory cytokine IL-33 by a NOD1-dependent pathway; this was confirmed in primary GECs from wild type (WT) and *Nod1*-deficient mice.[15] Similar to that, the human AGS cells transfected with shRNA specific for *NOD1* or with a *NOD1* knockout using CRISPR/Cas9 technology failed to produce IL-33 in response to *H. pylori*.[15] As expected, the NOD1-dependent production of IL-33 was orchestrated by a functional T4SS.[15] At 8 weeks postinfection, WT mice, but not $Nod1^{-/-}$ mice, exhibited a significant increase in *Il33* mRNA expression and IL-33 concentration in the gastric mucosa;[15] this suggests that NOD1-driven IL-33 may protect against *H. pylori*-mediated inflammatory responses thus favoring the persistence of the bacterium in the stomach.

Of all the hormones produced in the stomach, gastrin plays a major function in cellular processes involved in carcinogenesis such as proliferation, apoptosis, angiogenesis, motility, and tumor cell invasion. Hence, transgenic mice overexpressing gastrin under the transcriptional control of the insulin promoter (INS-GAS mice) rapidly develop gastric malignancies after *H. pylori* infection.[16] Gunawardhana et al found that the induction of the gene encoding gastrin by *H. pylori* is abolished in the presence of AG1478, an inhibitor of epidermal growth factor receptor (EGFR) in AGS cells.[17] Using siRNAs to specifically knockdown various signaling molecules, these authors demonstrated that *H. pylori* first induces the release of heparin-binding epidermal growth factor-like growth factor (HB-EGF), which in turn signals via Raf-1 proto-oncogene, serine/threonine kinase (RAF1), mitogen-activated protein kinase kinase 1 (MAP2K1, previously known as MEK1), and mitogen-activated protein kinase 1 (MAPK1, also called ERK2) to stimulate the transcription of the gene encoding gastrin.[17] In the same way, it has been already reported that there is increased EGFR phosphorylation in the *H. pylori*-induced human gastric inflammation and cancer development.[18] This year, the Wilson group also showed that treatment of *H. pylori*-infected INS-GAS mice or Mongolian gerbils with the EGFR inhibitor gefitinib resulted in (a) reduced activation of MAPK1/3 and activator protein 1 in GECs, (b) inhibition of chemokine synthesis by the gastric mucosa, (c) a decrease in myeloperoxidase-positive inflammatory cells, and (d) a marked reduction of gastric dysplasia and carcinoma.[19] Of particular interest, similar data were obtained in mice with epithelial cell-specific deletion of *Egfr* ($Egfr^{\Delta epi}$) and key findings were recapitulated using culture of primary GEC monolayers of WT and $Egfr^{\Delta epi}$ mice. Altogether, these data prove that the recruitment of myeloperoxidase-positive inflammatory cells to the infected gastric mucosa is orchestrated by epithelial EGFR. The same group had already demonstrated that EGFR signaling in myeloid cells is also involved in *H. pylori*-mediated mucosal immune response.[20] In this context, the inhibition of EGFR may represent an interesting chemopreventive strategy to alter the development of a persistent mucosal immune response, which may lead to carcinogenesis.

1.3 | Beyond epithelial cells

Although the gastric epithelium is the primary site of *H. pylori* colonization, *H. pylori* can access deeper tissues and the bacterium has been found in the vicinity of endothelial cells in the gastric submucosa.[21] By examining a previously unappreciated role of endothelial cells in eliciting a strong CXCL8 response against *H. pylori* infection, recent studies provide important new insights into the pathogenesis of *H. pylori*-associated gastric diseases. This last year, Tafreshi et al showed that, apart from gastric epithelial cells, endothelial cells may also contribute to *H. pylori*-induced inflammation.[22] They demonstrated that primary human umbilical vein cells (HUVEC) from an immortalized HUVEC cell line (EA.Hy926) produce more CXCL8 than the human GEC line AGS when stimulated with *H. pylori*.[22] This induction was completely inhibited when mutant strains lacking the complete *cag*PAI or the *cag*L gene, but not the *cag*A gene, were used to stimulate the cells; moreover, a recombinant CagL protein, but not a recombinant protein with a mutation in the arginine-glycine-aspartate (RGD) motif that is involved in the binding to the human transmembrane receptor integrin α5β1 and can activate EGFR,[23] also stimulates CXCL8 secretion by HUVECs.[22] At last, CXCL8 and IL-6 production by *H. pylori*-infected HUVECs has been significantly reduced with the EGFR inhibitor AG1478.[22] Thus, this work demonstrates that endothelial cells may play a critical role in the initiation and chronicity of the proinflammatory response in *H. pylori*-infected patients.

Urease displays an important function in *H. pylori* colonization and survival in the acidic environment of the stomach. Previous data have shown that, in a nonenzymatic way, *H. pylori* urease activates neutrophils to produce reactive oxygen species and also induces platelet aggregation, requiring ADP secretion modulated by the 12-lipoxygenase pathway, a signaling cascade also triggered by the physiological agonist collagen. Thus, Scopel-Guerra et al aimed to elucidate the mechanism underlying the activation of platelets exposed to an enzymatically active *H. pylori* urease holoenzyme and to its subunits UreA and UreB. For this purpose, they used recombinant versions of *H. pylori* urease and its two subunits, and they conducted platelet aggregation assays, flow cytometry and quantitative PCR to analyze how these proteins affect the platelets' physiology. They demonstrated that the *H. pylori* urease, and notably its B subunit, activates platelet aggregation and that the activation of platelets by *H. pylori* urease appears to lead these cells to exhibit a proinflammatory phenotype.[24] Therefore, platelets might possibly contribute to the development and/or the persistence of *H. pylori*-induced inflammation.

2 | IMMUNOLOGY

2.1 | Activation and role of myeloid cells

Neutrophils, monocytes and macrophages, and dendritic cells are the first cells recruited in the gastric lamina propria during *H. pylori* infection. A remaining topic is the identification of the cellular mechanism by which myeloid cells are recruited to the gastric mucosa and are activated by *H. pylori*. In their recent work, Arnold et al observed that the recruitment of macrophages and $CD11b^+$ dendritic cells (DCs), but not neutrophils and $CD4^+$ T cells, is inhibited in mice lacking C-C Motif Chemokine Receptor 2 (CCR2), a receptor for monocyte chemoattractant protein-1.[25] In addition, the recruited gastric macrophages and

DCs were found to express the receptor for fractalkine C-X3-C motif chemokine receptor 1 (CX3CR1), which may also play a role in myeloid cell recruitment.[25] Then, in an elegant study using an *H. pylori* strain that expresses the red fluorescent protein (RFP) and mice with a reconstituted human immune system, they showed that $CX3CR1^+$ macrophages and DCs phagocytose *H. pylori* in the gastric mucosa;[25] this result is particularly important because it demonstrates that myeloid cells are in contact not only with *H. pylori* soluble factors, but also with live bacteria during the infection, thus providing yet another strong rationale for the study of *H. pylori*/myeloid cell interactions. These $CX3CR1^+$/*H. pylori*$^+$ myeloid cells expressed high levels of the inflammasome nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3), and deletion of the gene encoding this protein led to a reduction of $FOXP3^+$ Treg response, to a concomitant increased gastric T helper (Th) 1 response, and to a decreased colonization of the gastric tissue by *H. pylori*.[25] The differentiation and the recruitment of DCs were not dependent on other inflammasome-related factors such as apoptosis-associated speck-like protein containing a carboxy-terminal CARD or caspase-1. Hence, the authors concluded that *H. pylori*-induced NLRP3 is required to generate Treg response independently of the role of the inflammasome.[25] This cellular event could be considered as a novel pathway of immune escape by *H. pylori*. In parallel, it was reported that NLRP3 mRNA expression and IL-1β secretion is increased in the human monocytic cell line THP-1 and blood-derived monocytes/macrophages infected with *H. pylori* or with mutants lacking functional T4SS, CagA, the vacuolating cytotoxin A (VacA), Cgt, or flagellin,[26] suggesting that other factors than the typical *H. pylori* virulence factors are involved in the stimulation of the inflammasome in humans.

MicroRNAs (miRNAs) are short noncoding RNAs (20–22 nt) that inhibit mRNA transduction and induce their degradation. Pagliari et al were able to identify the miRNAs induced by *H. pylori* in human macrophages differentiated from blood-derived monocytes using colony stimulating factor 1 (CSF1 or MCSF). Using miRNA microarray, the authors observed 55 downregulated and 46 upregulated miRNAs after 24 h of infection.[27] Using a sophisticated comparative mapping of the miRNAs inhibited by *H. pylori* and of the mRNAs induced during the infection, they found that the downregulation of miR-4270 was correlated with a marked overexpression of the activating receptor CD300E, which is involved in the inducible expression of IL-1β and IL-6, and in the inhibition of MHC-II-dependent pathway of antigen presentation in human macrophages.[27] Thus, these findings highlight a novel mechanism by which *H. pylori* elicits persistent inflammation and escapes T-cell-dependent specific immune response.

2.2 | The adaptive immune responses

$CD4^+$ Th cells are central to many aspects of immune response. The proinflammatory response characterized by the Th1 and Th17 subtypes contributes to protection against *H. pylori* via cytokine secretion and activation of effector cells, but also supports chronic inflammation and injury that can ultimately lead to development of gastric cancer.[28] In the last year, clinical investigations emphasized that Th1 and Th17 responses are elicited during *H. pylori* infection. Adamsson et al reported that the level of expression of the genes encoding the prototype Th17 cytokine IL-17A and the Th1 lymphokine IFN-γ is enhanced

in the corpus and antrum of Swedish patients with *H. pylori* infection compared to uninfected individuals;[29] more importantly they confirmed by immunofluorescence that the number of IL-17A$^+$ and IFN-γ^+ cells is also increased in *H. pylori*-infected patients.[29] Similar to that, a significant increase in *IL17A* mRNA transcripts was observed in the gastric tissue of *H. pylori*-infected patients from Iran[30] and France[31] compared to uninfected ones. Arachchi et al also reported that *H. pylori* infection is associated with elevated IL-17 serum concentrations in a human cohort from Sri-Lanka.[32] As expected, the severity of the gastritis was positively correlated with the production of IL-17.[32] However, two studies showed that the number of infiltrating Th17 cells is higher in *H. pylori*-infected patients with peptic ulcers compared to individuals with *H. pylori* gastritis.[29,30] To converse, Cremniter et al described that the same level of *IL17A* mRNA is found in patients with gastritis, ulcer, or precancerous and cancerous lesions.[31]

An alternative picture to the Th1/Th17 synergy and antagonism emerged from a study by D'Souza et al These authors assessed the induction and the role of mucosal-associated invariant T (MAIT) cells during *H. pylori* infection; these cells expressed the MHC class I-related gene protein (MR1), were activated by riboflavin-producing bacteria, secreted IFN-γ and IL-17, and displayed a protective role in bacterial infections.[33,34] They found that MAIT cells were enhanced in the gastric mucosa of patients or mice infected with *H. pylori*.[35] Transgenic *Vα19iCα*$^{-/-}$*Mr1*$^{+/+}$ mice, which express only the TCRα chain (Vα19i) harbored by MAIT cells,[36] exhibited high levels of MAIT cells in the stomach and showed increased gastritis compared to *Vα19iCα*$^{-/-}$*Mr1*$^{-/-}$ mice upon *H. pylori* infection.[35] Similar results were obtained in animals with a boosted MAIT cell levels using preinfection with *Salmonella enterica* serovar *Typhimurium* or pretreatment with Pam2Cys plus 5-(2-oxopropylideneamino)-6-d-ribit ylaminouracil. In an important way, these last animals infected with *H. pylori* exhibited not only more gastric T cells, but also an increase in neutrophils, macrophages, eosinophils, and dendritic cells. These data emphasize that MAIT cells should be taken into consideration to elucidate of the immunopathology of *H. pylori* infection; however, the level of bacterial colonization in mice with increased MAIT cells was not provided in this article, so we are not able to estimate the effect of MAIT cells on *the H. pylori* burden.[36]

3 | VACCINES

During the past year, studies sought to identify *H. pylori* antigens and epitopes that may lead to the development of efficient vaccine protocols were performed. By identifying the immunodominant antigens of 30 different groups of *H. pylori* proteins, based on their molecular weight, Sun et al determined that inosine 5′-monophosphate dehydrogenase, type II citrate synthase, and urease subunit beta are three protective antigens inducing dominant Th1 and Th17 responses.[37] Moreover, immunization of mice with recombinant *H. pylori* FliD protein was shown to elicit strong Th1 and Th17 immune responses and a significant reduction in *H. pylori* bacterial load by more than a 2-log order compared to mice treated with the adjuvant only.[38] At last, Li et al identified that only the epitopes $UreB_{373–385}$ and $UreB_{317–329}$ are effective in stimulating a strong T-cell response and are protective against *H. pylori* challenge in mice.[39]

More particularly, Guo et al developed multivalent epitope-based vaccines, called CWAE vaccines. They cloned in *Escherichia coli* two plasmids containing $CD4^+$ Th cell and B cell epitopes in antigenic *H. pylori* proteins predicted by T Cell Epitope Prediction Tools and B Cell Epitope Prediction Tools. The first harbored (a) two molecular adjuvants (mucosal adjuvant cholera toxin B subunit and Th1-type adjuvant), (b) tandem copies of the selected B and Th cell epitopes ($UreA_{27–53}$, $UreA_{183–203}$, $HpaA_{132–141}$, and heat shock protein $60_{189–203}$), and (c) epitope-rich regions $UreB_{158–251}$ containing four known Th or B cell epitopes and $UreB_{321–385}$ containing three Th or B cell epitopes.[40] The second was composed of (a) the mucosal adjuvant cholera toxin B subunit, (b) Th and B-cell epitopes ($UreA_{27–90}$, $UreA_{183–225}$, $UreB_{327–385}$, membrane-associated lipoprotein $Lpp20_{58–97}$, $HpaA_{52–194}$, and $CagL_{21–139}$), and (c) an Lpp20 mimotope.[41] These proteins were administered *per os* together with aluminum hydroxide adjuvant or polysaccharide adjuvant in gerbils infected with *H. pylori* SS1, 6 weeks after infection; *H. pylori* burden was assessed 5 weeks after vaccination. Stomach colonization was reduced by 3–4-log orders in animals treated with both CWAE vaccines compared to infected gerbils and by 1-log versus gerbils vaccinated with either a univalent epitope-based vaccine against *H. pylori* urease[40] or a urease vaccine.[41] Antibody response and gastritis were also improved with the CWAE vaccine.[40] Thus, this multivalent epitope-based vaccine may be a promising candidate to control *H. pylori* infection.

As an alternative to classical vaccines, two groups engineered recombinant *Lactococcus lactis* strains to overexpress *H. pylori* antigens: (a) the complete *H. pylori* adhesin A (HpaA),[42] a lipoprotein located in the cellular outer membrane and flagellum sheath, and (b) the neutrophil-activating protein A subunit (NapA),[43] a virulence factor that recruits and stimulates neutrophils and protects *H. pylori* against oxidative damage.[44] Animals inoculated once a week for four weeks with the recombinant *L. lactis* strain developed anti-HpaA and anti-NapA IgG, and fecal IgA.[42,43] The immunization with the NapA-expressing *L. lactis* strain also initiated a Th1 and Th17 response by splenic cells under an antigenic recall assay using *H. pylori* lysates, a partial reduction in *H. pylori* colonization,[43] but no significant change in the level of gastritis. It would now be of interest to assess the role of the HpaA-expressing strain on *H. pylori* colonization and clinical outcomes.

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REFERENCES

1. Li N, Tang B, Jia YP, et al. *Helicobacter pylori* CagA protein negatively regulates autophagy and promotes inflammatory response via c-Met-PI3K/Akt-mTOR signaling pathway. Front Cell Infect Microbiol 2017;7:417. [PubMed: 28983474]

2. Moynagh PN. The Pellino family: IRAK E3 ligases with emerging roles in innate immune signalling. Trends Immunol 2009;30:33–42. [PubMed: 19022706]

3. Schauvliege R, Janssens S, Beyaert R. Pellino proteins are more than scaffold proteins in TLR/IL-1R signalling: a role as novel RING E3-ubiquitin-ligases. FEBS Lett 2006;580:4697–4702. [PubMed: 16884718]

4. Yokota S, Ohnishi T, Muroi M, Tanamoto K, Fujii N, Amano K. Highly-purified *Helicobacter pylori* LPS preparations induce weak inflammatory reactions and utilize Toll-like receptor 2 complex but not Toll-like receptor 4 complex. FEMS Immunol Med Microbiol 2007;51:140–148. [PubMed: 17645528]

5. Gobert AP, Wilson KT. Human and *Helicobacter pylori* interactions determine the outcome of gastric diseases. Curr Top Microbiol Immunol 2017;400:27–52. [PubMed: 28124148]

6. Smith SM, Freeley M, Moynagh PN, Kelleher DP. Differential modulation of *Helicobacter pylori* lipopolysaccharide-mediated TLR2 signaling by individual Pellino proteins. Helicobacter. 2017;22:e12325.

7. Stein SC, Faber E, Bats SH, et al. *Helicobacter pylori* modulates host cell responses by CagT4SS-dependent translocation of an intermediate metabolite of LPS inner core heptose biosynthesis. PLoS Pathog 2017;13:e1006514. [PubMed: 28715499]

8. Gall A, Gaudet RG, Gray-Owen SD, Salama NR. TIFA signaling in gastric epithelial cells initiates the Cag type 4 secretion system-dependent innate immune response to *Helicobacter pylori* Infection. MBio 2017;8:e01168–17. [PubMed: 28811347]

9. Zimmermann S, Pfannkuch L, Al-Zeer MA, et al. ALPK1-and TIFA-dependent innate immune response triggered by the *Helicobacter pylori* yype IV secretion system. Cell Rep 2017;20:2384–2395. [PubMed: 28877472]

10. Feige MH, Vieth M, Sokolova O, Tager C, Naumann M. *Helicobacter pylori* induces direct activation of the lymphotoxin beta receptor and non-canonical nuclear factor-kappa B signaling. Biochim Biophys Acta 2018;1865:545–550.

11. Viala J, Chaput C, Boneca IG, et al. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. Nat Immunol 2004;5:1166–1174. [PubMed: 15489856]

12. Varga MG, Shaffer CL, Sierra JC, et al. Pathogenic *Helicobacter pylori* strains translocate DNA and activate TLR9 via the cancer-associated cag type IV secretion system. Oncogene. 2016;35:6262–6269. [PubMed: 27157617]

13. Wunder C, Churin Y, Winau F, et al. Cholesterol glucosylation promotes immune evasion by *Helicobacter pylori*. Nat Med 2006;12:1030–1038. [PubMed: 16951684]

14. Morey P, Pfannkuch L, Pang E, et al. *Helicobacter pylori* depletes cholesterol in gastric glands to prevent interferon gamma signaling and escape the inflammatory response. Gastroenterology. 2018;154:1391–1404. [PubMed: 29273450]

15. Tran LS, Tran D, De Paoli A, et al. NOD1 is required for *Helicobacter pylori* induction of IL-33 responses in gastric epithelial cells. Cell Microbiol 2018;20:e12826. [PubMed: 29392836]

16. Fox JG, Rogers AB, Ihrig M, et al. *Helicobacter pylori*-associated gastric cancer in INS-GAS mice is gender specific. Cancer Res 2003;63:942–950. [PubMed: 12615707]

17. Gunawardhana N, Jang S, Choi YH, et al. *Helicobacter pylori*-induced HB-EGF upregulates gastrin expression via the EGF receptor, C-Raf, Mek1, and Erk2 in the MAPK pathway. Front Cell Infect Microbiol 2017;7:541. [PubMed: 29379775]

18. Chaturvedi R, Asim M, Piazuelo MB, et al. Activation of EGFR and ERBB2 by *Helicobacter pylori* results in survival of gastric epithelial cells with DNA damage. Gastroenterology. 2014;146:1739–1751. [PubMed: 24530706]

19. Sierra JC, Asim M, Verriere TG, et al. Epidermal growth factor receptor inhibition downregulates *Helicobacter pylori*-induced epithelial inflammatory responses, DNA damage and gastric carcinogenesis. Gut 2017;7:1247–1260.

20. Hardbower DM, Singh K, Asim M, et al. EGFR regulates macrophage activation and function in bacterial infection. J Clin Invest 2016;126:3296–3312. [PubMed: 27482886]

21. Necchi V, Candusso ME, Tava F, et al. Intracellular, intercellular, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by *Helicobacter pylori*. Gastroenterology. 2007;132:1009–1023. [PubMed: 17383424]

22. Tafreshi M, Guan J, Gorrell RJ, et al. *Helicobacter pylori* type IV secretion system and its adhesin subunit, CagL, mediate potent inflammatory responses in primary human endothelial cells. Front Cell Infect Microbiol 2018;8:22. [PubMed: 29468142]
23. Kwok T, Zabler D, Urman S, et al. *Helicobacter* exploits integrin for type IV secretion and kinase activation. Nature. 2007;449:862–866. [PubMed: 17943123]
24. Scopel-Guerra A, Olivera-Severo D, Staniscuaski F, et al. The impact of *Helicobacter pylori* urease upon platelets and consequent contributions to inflammation. Front Microbiol 2017;8:2447. [PubMed: 29312166]
25. Arnold IC, Zhang X, Urban S, et al. NLRP3 controls the development of gastrointestinal CD11b(+) dendritic cells in the steady state and during chronic bacterial infection. Cell Rep 2017;21:3860–3872. [PubMed: 29281833]
26. Pachathundikandi SK, Backert S. *Helicobacter pylori* controls NLRP3 expression by regulating hsa-miR-223–3p and IL-10 in cultured and primary human immune cells. Innate Immun 2018;24:11–23. [PubMed: 29145789]
27. Pagliari M, Munari F, Toffoletto M, et al. *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. [PubMed: 29085364]
28. Jafarzadeh A, Larussa T, Nemati M, Jalapour S. T cell subsets play an important role in the determination of the clinical outcome of *Helicobacter pylori* infection. Microb Pathog 2018;116: 227–236. [PubMed: 29407232]
29. Adamsson J, Ottsjo LS, Lundin SB, Svennerholm AM, Raghavan S. Gastric expression of IL-17A and IFNgamma in *Helicobacter pylori* infected individuals is related to symptoms. Cytokine. 2017;99:30–34. [PubMed: 28683359]
30. Bagheri N, Razavi A, Pourgheysari B, et al. Up-regulated Th17 cell function is associated with increased peptic ulcer disease in *Helicobacter pylori*-infection. Infect Genet Evol 2018;60: 117–125. [PubMed: 29481961]
31. Cremniter J, Bodet C, Tougeron D, et al. Th-17 response and antimicrobial peptide expression are uniformly expressed in gastric mucosa of *Helicobacter pylori*-infected patients independently of their clinical outcomes. Helicobacter. 2018;23:e12479. [PubMed: 29582503]
32. Arachchi PS, Fernando N, Weerasekera MM, Senevirathna B, Weerasekera DD, Gunasekara CP. Proinflammatory cytokine IL-17 shows a significant association with *Helicobacter pylori* infection and disease severity. Gastroenterol Res Pract 2017;2017:6265150. [PubMed: 29391865]
33. Meierovics A, Yankelevich WJ, Cowley SC. MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. Proc Natl Acad Sci USA 2013;110:E3119–E3128. [PubMed: 23898209]
34. Le Bourhis L, Martin E, Peguillet I, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 2010;11:701–708. [PubMed: 20581831]
35. D'Souza C, Pediongco T, Wang H, et al. Mucosal-associated invariant t cells augment immunopathology and gastritis in chronic *Helicobacter pylori* infection. J Immunol 2018;200:1901–1916. [PubMed: 29378910]
36. Kawachi I, Maldonado J, Strader C, Gilfillan S. MR1-restricted V alpha 19i mucosal-associated invariant T cells are innate T cells in the gut lamina propria that provide a rapid and diverse cytokine response. J Immunol 2006;176:1618–1627. [PubMed: 16424191]
37. Sun H, Yuan H, Tan R, et al. Immunodominant antigens that induce Th1 and Th17 responses protect mice against *Helicobacter pylori* infection. Oncotarget. 2018;9:12050–12063. [PubMed: 29552292]
38. Ghasemi A, Mohammad N, Mautner J, Karsabet MT, Ardjmand A, Moniri R. Immunization with recombinant FliD confers protection against *Helicobacter pylori* infection in mice. Mol Immunol 2018;94:176–182. [PubMed: 29324238]
39. Li B, Yuan H, Chen L, et al. The influence of adjuvant on UreB protection against *Helicobacter pylori* through the diversity of CD4 + T-cell epitope repertoire. Oncotarget. 2017;8:68138–68152. [PubMed: 28978104]

40. Guo L, Yang H, Tang F, et al. Oral Immunization with a multivalent epitope-based vaccine, based on NAP, Urease, HSP60, and HpaA, provides therapeutic effect on H. pylori Infection in Mongolian gerbils. Front Cell Infect Microbiol 2017;7:349. [PubMed: 28824883]

41. Guo L, Yin R, Xu G, et al. Immunologic properties and therapeutic efficacy of a multivalent epitope-based vaccine against four *Helicobacter pylori* adhesins (urease, Lpp20, HpaA, and CagL) in Mongolian gerbils. Helicobacter. 2017;22:e12428.

42. Zhang R, Wang C, Cheng W, et al. Delivery of *Helicobacter pylori* HpaA to gastrointestinal mucosal immune sites using *Lactococcus lactis* and its immune efficacy in mice. Biotechnol Lett 2018;40:585–590. [PubMed: 29299716]

43. Peng X, Zhang R, Duan G, et al. Production and delivery of *Helicobacter pylori* NapA in *Lactococcus lactis* and its protective efficacy and immune modulatory activity. Sci Rep 2018;8:6435. [PubMed: 29691472]

44. Gobert AP, Wilson KT. Polyamine-and NADPH-dependent generation of ROS during *Helicobacter pylori* infection: a blessing in disguise. Free Radic Biol Med 2017;105:16–27. [PubMed: 27682363]