cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Induction and Regulation of the Innate Immune Response in *Helicobacter pylori* Infection

Alain P. Gobert[1,2,3] and Keith T. Wilson[1,2,3,4,5]

[1]Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, [2]Center for Mucosal Inflammation and Cancer, [3]Program in Cancer Biology, [4]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee; and [5]Veterans Affairs Tennessee Valley Healthcare System, Nashville, Tennessee.

SUMMARY

Helicobacter pylori is a pathogen that causes gastric cancer by stimulating inflammation. The immune response of epithelial cells and myeloid cells to *H. pylori* is regulated by the gastric microbiota and individual genetic susceptibility, which determine the outcome of the disease.

Gastric cancer (GC) is the fifth most common cancer and the fourth most common cause of cancer-related death worldwide. The intestinal type of GC progresses from acute to chronic gastritis, multifocal atrophic gastritis, intestinal metaplasia, dysplasia, and carcinoma. Infection of the stomach by *Helicobacter pylori*, a Gram-negative bacterium that infects approximately 50% of the world's population, is the causal determinant that initiates the gastric inflammation and then disease progression. In this context, the induction of the innate immune response of gastric epithelial cells and myeloid cells by *H. pylori* effectors plays a critical role in the outcome of the infection. However, only 1% to 3% of infected patients develop gastric adenocarcinoma, emphasizing that other mechanisms regulate the localized non-specific response, including the gastric microbiota and genetic factors. This review summarizes studies describing the factors that induce and regulate the mucosal innate immune response during *H. pylori* infection. *(Cell Mol Gastroenterol Hepatol 2022; 13:1347–1363; https://doi.org/10.1016/j.jcmgh.2022.01.022)*

Keywords: Gastric Cancer; Gastritis; Microbiota; Polymorphism; Virulence Factors.

Helicobacter pylori is a Gram-negative bacterium that colonizes the stomach of 4.4 billion people.[1] Its persistence in the stomach causes chronic gastritis, which can progress to intestinal metaplasia (IM), and gastric adenocarcinoma,[2] the fourth leading cause of cancer mortality.[3] Over 100,000 years of coevolution between *Helicobacter pylori* and humans[4] has shaped the mucosal immune system. A fragile equilibrium enables the pathogen to escape antibacterial immunity to survive, or allows the host to control the infection and limit damage from inflammation. This crosstalk is regulated at different levels. First, a myriad of *H. pylori* factors induces and controls the non-specific response of gastric epithelial cells (GECs) and immune cells. Second, the gastric microbiota influences the innate signaling. Third, host gene polymorphisms have an impact of *H. pylori*-mediated disease outcome. These 3 topics are covered in this review.

Induction of Innate Responses in GECs by H. pylori Effectors

Type 4 Secretion System: CagA and Beyond

Most *H. pylori* strains that induce peptic ulcers or neoplastic transformation possess the cytotoxin-associated gene (*cag*) pathogenicity island (*cag*PAI), which carries genes encoding for a functional type 4 secretion system (T4SS) and the virulence factor CagA.[5–7] CagA is injected into the cytoplasm of GECs by the T4SS,[7] phosphorylated on tyrosine residues of Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs by SRC and then ABL kinases,[8–10] and binds/activates SHP2,[11] which signals through ERK1/2 to induce cytoskeletal rearrangements and increased motility linked to carcinogenesis.[12,13] Native CagA can also modulate cellular functions.[14] Large epidemiological studies have correlated *cagA*$^+$ strains to higher rates of gastric cancer (GC).[15,16]

Numerous studies have shown that multiple GEC lines produce more interleukin (IL)-8 when stimulated with *H. pylori* strains harboring the *cag*PAI compared with *cag*PAI$^-$ isolates.[17–20] Using cDNA arrays, it was shown that in addition to IL-8, immune genes encoding for IL-2, IL-5, and tumor necrosis factor-alpha (TNF-α), for example, are less expressed in KATO-3 cells infected with *cag*PAI$^-$ compared with *cag*PAI$^+$ isolates.[21] Thus, effectors encoded by the *cag*PAI have been implicated in the induction of innate responses by GECs.

Abbreviations used in this paper: **cag, cytotoxin-associated gene; cagPAI, cag pathogenicity island; DC, dendritic cell; GC, gastric cancer; GECs, gastric epithelial cells; GF, germ-free; Hsp, heat shock protein; IL, interleukin; IM, intestinal metaplasia; INS-GAS, insulin-gastrin; Jax, Jackson Laboratory; LPS, lipopolysaccharide; NF-κB, nuclear factor kappa B; OipA, outer inflammatory protein A; PG, peptidoglycan; Tac, Taconic Bioscience; T4SS, type 4 secretion system; TNF, tumor necrosis factor; WT, wild-type.**

Most current article

© 2022 The Authors. Published by Elsevier Inc. on behalf of the AGA Institute. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2352-345X
https://doi.org/10.1016/j.jcmgh.2022.01.022

H. pylori mutants for *cagE*, encoding for an ATPase of the T4SS, lose their ability to stimulate ERK1/2, p38 and JNK phosphorylation,[22] nuclear factor kappa B (NF-κB) NF-κB activation,[19] and to induce IL-8[5,6,23] or other chemokines, such as CCL2 or CCL5.[24] Primary human GECs, which synthesized higher levels of IL-8 than MKN-28 cells in response to *H. pylori* strain TN2F4, produce less chemokines in response to an isogenic *cagE* mutant compared with the wild-type (WT) strain.[25] Deletion of one of the *cag*PAI genes involved in T4SS structure or function is sufficient to reduce the production of IL-8.[5,6,26,27] Also, *cag*PAI$^+$ strains induce greater phosphorylation of epidermal growth factor receptor than *cag*PAI$^-$ strains in AGS cells, and a *cagE* mutant did not activate epidermal growth factor receptor.[28] Thus, a functional T4SS is required for full stimulation of innate responses in GECs.

Therefore, the question is which *H. pylori* effectors injected by the T4SS stimulate innate responses (Figure 1). Transfection of AGS cells with a plasmid encoding CagA stimulates IL-8 production through SRC/ERK1/2/NF-κB.[29] Moreover, we found that expression of *CXCL8* mRNA is reduced by more than 60% in AGS cells infected for 3 hours with *H. pylori* 60190 lacking the *cagA* gene compared with the parental strain.[30] Similarly, AKT1-MTOR-S6K signaling and formation of the EEF1A1-PKCδ-STAT3 complex, leading to synthesis of IL-6, is rapidly activated in *H. pylori*-infected AGS cells and human primary GECs through a CagA-dependent pathway.[31,32] In contrast, when GECs are infected for a longer period (6–24 hours), the levels of *CXCL8* mRNA are similar between cells infected with WT or *cagA*$^-$ *H. pylori*.[18,19,23,26,30,33] Interestingly, Brandt et al identified 2 distinct groups of *H. pylori* carrying a functional *cag*PAI: the high and the low IL-8 inducers.[34] The *cagA* mutants of the high-inducer strains induced significantly less IL-8 than WT *H. pylori*, whereas deletion of *cagA* in the low-inducer group had no effect on their IL-8-inducing ability.[34] Importantly, a *cagA* mutant constructed in a low-inducer strain and complemented with a *cagA* gene from a high-inducer group stimulated high levels of IL-8 production, and vice versa;[34] this was associated with CagA-mediated stimulation of the RAS/ERK1/2/NF-κB pathway.[34]

Further, it has been reported that CagA phosphorylation is required to activate innate responses of GECs.[6,29,30,34] Although the number of EPIYA motifs, and thus CagA phosphorylation level, has been reported to have no effect on IL-8 secretion,[20] coculture of AGS cells with Western strains from Scotland expressing CagA with ABC motifs or with East Asian strains from China expressing ABD motifs evidenced that East Asian strains induced significantly more IL-8 secretion than ABC strains.[35] This suggests that East Asian *H. pylori* strains, associated with high levels of atrophic gastritis and GC, may induce more inflammation within the stomach. Lastly, 2 studies have highlighted that CagA and pCagA exhibit different effects on GECs: First, Suzuki et al showed that pCagA is required for IL-8 production by infected AGS cells in the early stage of the infection, and then both pCagA and a conserved motif in the C-terminal region of CagA, called conserved repeat responsible for phosphorylation-independent activity, are implicated in late cell activation;[36] second, Lee et al found that the formation of the GP130/CagA/SHP2 complex stimulates the JAK2/STAT3 signaling pathway, whereas the same complex with pCagA preferentially activates the ERK1/2 pathway.[37] Thus, it appears that CagA/pCagA is involved in activation of GECs, but that maximal innate response requires other effectors translocated through the T4SS.

Using HEK293 cells transfected with an NF-κB-luciferase reporter construct and a dominant negative plasmid for *NOD1*, Viala et al observed that *H. pylori* and a *cagA* mutant, but not a *cagM*-deficient strain, activate NF-κB through NOD1.[38] NF-κB induction and IL-8 production are reduced when AGS cells are stimulated with a mutant deficient in lytic trans-glycosylase activity (D*slt*) that releases less peptidoglycan (PG) muropeptides than WT *H. pylori*.[38] The authors concluded that the T4SS allows for PG delivery in GECs to stimulate NOD1-dependent signaling (Figure 1). Similar findings were observed with an *H. pylori* mutant lacking the gene *pgdA* that encodes a peptidoglycan deacetylase.[39] This mechanism was also implicated in stimulation of MAP kinases p38 and ERK1/2, and the transcription factor AP-1,[40] and induction of beta-defensin 2 expression by *H. pylori*-infected AGS cells.[41] Interestingly, induction of IL-3 by *H. pylori* in AGS cells depends on both NOD1 signaling and CagA translocation,[42] demonstrating that *H. pylori* can exploit its T4SS to stimulate various pathways simultaneously. Note that *H. pylori* outer membrane vesicles, irrespective of their *cag*PAI status, can also deliver PG in GECs and induce NOD1 signaling[43] and NOD1/RIP2-mediated autophagy.[44]

Increased colonization by *cag*PAI$^+$ strains was reported in *Nod1*$^{-/-}$ mice compared with WT animals at 7 and 30 days post-inoculation.[38] Of importance, the colonization by *H. felis*, a bacterium lacking a *cag*PAI homolog, or with *H. pylori cagM*$^-$ was similar in both genotypes of mice, demonstrating the essential role of the T4SS in PG signaling in vivo, although gastritis severity was not described in this paper.[38] However, C57BL/6 and FVB/N transgenic insulin-gastrin (INS-GAS) mice with deletion of *Nod1* exhibited increased gastritis at 20 days post-infection, but not after 90 days, and there was no effect on colonization.[45] These authors also observed spontaneous dysplasia in INS-GAS mice with *Nod1* deletion, which was further increased by *H. pylori* infection.[45] In contrast, infection of Mongolian gerbils with *H. pylori* D*pgdA*, which fails to acetylate PG and induces less NF-κB and IL-8 production by AGS cells, results in significantly decreased levels of inflammation and malignant lesions in the stomach.[39] Therefore, efforts are still needed to decipher the role of the T4SS/PG/NOD1 signaling pathway in *H. pylori*-mediated diseases.

Additionally, using HEK293 cells transfected with a TLR9 expression plasmid and an NF-κB/AP-1-linked reporter, Varga et al found that *H. pylori* and a *cagA*-deficient strain, but not mutants for the major constituents of the T4SS, can signal through TLR9 to induce chemokine synthesis,[46] evidencing that the T4SS can also be used by *H. pylori* to

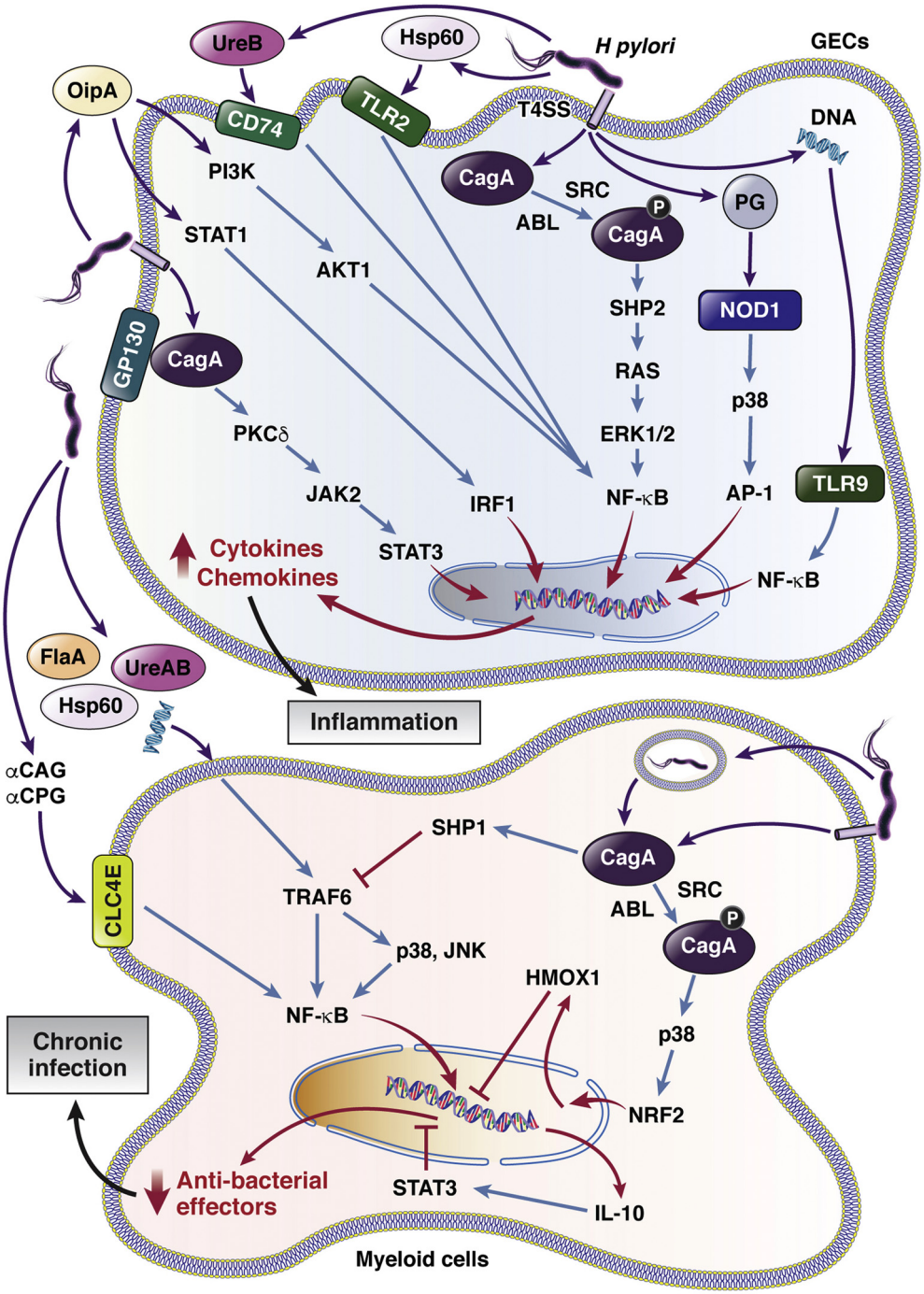

Figure 1. Immunopathogenesis of *H. pylori* infection. Numerous factors from *H. pylori*, notably native or phosphorylated CagA, stimulate a strong pro-inflammatory response, characterized by the production of high levels of cytokines and chemokines in GECs. This leads to inflammation and disease progression. Although myeloid cells respond to bacterial proteins and metabolites, the main effect of CagA is to dampen the innate response in the early stage of the infection, allowing *H. pylori* persistence. aCAG, cholesteryl acyl a-glucoside; aCPG, cholesteryl phosphatidyl a-glucoside; CLC4E, macrophage-inducible C-type lectin receptor.

inject DNA in GECs (Figure 1). Further, the ATPases Caga and CagE are essential for *H. pylori*-induced NF-κB activation and IL-8 secretion through TLR9.[27] A cagb mutant that cannot translocate CagA is still able to stimulate TLR9,[27] demonstrating that translocation of DNA and CagA requires different energetic process. Because TLR9 expression is exclusively observed at the basolateral localization of GECs during *H. pylori* gastritis,[47] injection of DNA can be considered as an adaptation of *H. pylori* to the response of the mucosa.

Importantly, monolayers of human gastroids infected with *H. pylori* show an increase of *CXCL8* transcripts as soon as 3 hours post-infection, and a multiplicity of infection-dependent production of IL-8 at 24 hours. However, compared with gastric cell lines, deletion of the *cag*PAI does not affect IL-8 production.[48] Further experiments with organoids are needed to determine the interaction of *H. pylori* with the innate response of human primary GECs.

VacA

VacA is a pore-forming toxin that stimulates vacuole formation in GECs and contributes to *H. pylori* pathogenesis. Numerous publications have established that VacA does not directly stimulate the pro-inflammatory response of GECs.[18,33,49] However, increased CagA-induced cellular elongations were observed when a *vacA* mutant was used to stimulate GECs, without affecting CagA phosphorylation, demonstrating that VacA dampens the effect of CagA on cells,[50] though it has not been shown that VacA itself suppresses the innate response of GECs. Nonetheless, VacA inhibits MTORC1 signaling in HEK293 and AGS cells by favoring its dissociation from the lysosomal surface.[51]

Urease

Urease catalyzes the hydrolysis of urea into ammonia and carbamate, which decomposes into ammonia and carbonic acid. Ammonia and carbonic acid are in equilibrium with their deprotonated and protonated forms, leading to an increase in pH. Thus, urease is essential to neutralize gastric acid and for *H. pylori* colonization.

Although Sharma et al reported that a urease mutant stimulates IL-8 secretion by GECs as the parental strain,[18] Tanahashi et al demonstrated that purified urease and the UreA subunit promotes production of IL-6 and TNF-α, but not IL-8, by MKN-45 cells.[52] In contrast, the UreB subunit directly binds to CD74 and stimulates NF-κB activation and IL-8 synthesis by N87 cells[53] (Figure 1). Notably, in these last 2 studies, the concentration of UreA and UreB used was very high (10 mg/mL); and although urease can be released by *H. pylori* using a secretion system[54] or by autolysis,[55] most of the enzyme remains intracellular within the bacterium and does not fully participate in GEC stimulation.

Flagellin

Flagellin is the protein subunit that polymerizes to form the flagella. Highly motile clinical strains induce more IL-8 production than bacteria with low motility.[56] This difference is likely due to increased adhesion of highly motile strains to GECs rather than a direct effect of flagellin; when GECs are overlaid with methylcellulose solution, which mimics the mucus layer and increase the velocity of the bacteria, the induction of IL-1α, IL-8, MCP-1, and granulocyte-macrophage colony-stimulating factor is increased.[56] Contrary to *Salmonella* typhimurium, *H. pylori* does not secrete flagellin and, therefore, a supernatant of *H. pylori* does not induce IL-8 production by AGS cells.[57] In addition, disruption of *H. pylori flaA* decreased motility, but had no effect on *H. pylori*-induced p38 phosphorylation and IL-8 secretion, and recombinant FlaA protein also fails to stimulate IL-8.[57] Smith et al showed that NF-κB activity was detected in *H. pylori*-infected HEK293 cells transfected with a plasmid leading to the expression of TLR5, which recognizes flagellin, and that silencing of *TLR5* in MKN45 GECs significantly reduced NF-κB activation;[58] however, production of IL-8, GRO-α, and MIP-3α was not observed,[58] which may suggest that *H. pylori* flagellin can signal in GECs, but is not sufficient for a complete response.

Heat Shock Protein

H. pylori heat shock protein (Hsp)60 is one of the main components representing the framework of the chaperone system. Using recombinant proteins generated in *Escherichia coli*, Yamaguchi *et al.* reported that long exposure of the gastric cell line KATO III[59] or human primary GECs[60] to *H. pylori* Hsp60 induces IL-8 secretion (Figure 1). This effect and the Hsp60-induced activation of NF-κB was inhibited by an anti-TLR2 antibody, and to a lesser extent by an anti-TLR4 antibody.[61]

Outer Membrane Proteins

The outer barrier of Gram-negative bacteria consists of the inner monolayer containing phospholipids and the outer monolayer mainly formed by outer membrane proteins. Outer inflammatory protein A (OipA) is a member of the Hop family, also referred to as HopH, mainly present in *cagA*$^+$ strains. Deletion of the *oipA* gene in isolates from Japanese and United States patients in which OipA is functional led to a 50% reduction of IL-8 production by GECs after 24 hours.[62] In contrast, a mutation in *H. pylori* in which OipA is not functional due to CT dinucleotide repeats in the 5′ region of the gene had no effect on IL-8-inducing activity.[62] Functional OipA is associated with high levels of *H. pylori* colonization, neutrophil infiltration, and mucosal IL-8 in humans[63] and mice.[64] Interestingly, CagA translocation and cytokine induction in AGS cells are not affected by *oipA* deletion after a 4-hour infection[65] or with a high multiplicity of infection (1000),[66] which may suggest that OipA is involved, but not essential, in induction of the innate response of GECs in later stages of infection.

Several studies have reported that OipA and the *cag*PAI have a complementary effect on activation of GECs: (1) phosphorylation of AKT1 at Ser473 and Thr308 is dependent on OipA and *cag*PAI, respectively, and the PI3K-AKT pathway is required for full activation of IL-8[67]; (2) whereas the *cag*PAI is the main inducer of AP-1 and NF-κB, OipA stimulates STAT1 phosphorylation and IRF-1 signaling, which are all required for the full activity of the *CXCL8* promoter region[68]; (3) *H. pylori*-induced RANTES/CCL5 gene transcription requires the presence of the interferon-stimulated responsive element, which is mainly stimulated by the OipA-p38 pathway, and *cag*PAI-dependent activation of NF-κB[69]; and (4) activation of p38 by OipA, and AP-1 and NF-κB by the *cag*PAI is required for IL-6 production by GECs.[70]

Lastly, other outer membrane proteins such as AlpAB[71] or HomB[72] exhibit the ability to stimulate IL-8 production by GECs, but their effect is minor compared with OipA (Figure 1).

Activation of Professional Immune Cells

In antral biopsies, expression of the genes encoding for IL-8 and GROα was detected more frequently in *H. pylori*-infected vs uninfected persons, and most IL-8 or GROα-positive cells within the lamina propria are $CD68^+$ macrophages.[73] This finding indicates that after the first interaction of *H. pylori* with GECs, long-term infection leads to activation of recruited immune cells and thus chronic inflammation.

Several *H. pylori* factors, including urease,[74] Hsp60,[75,76] flagellin,[77] NapA,[78] or DNA[79] have been reported to directly stimulate myeloid cells at a distance from *H. pylori*. This occurs through translocation of these effectors through the epithelial barrier or when epithelial damage allows *H. pylori* to invade the lamina propria.[80,81] In addition, Nagata et al recently reported that cholesteryl acyl a-glucoside and cholesteryl phosphatidyl a-glucoside synthesized by *H. pylori* from host cholesterol stimulates murine and human dendritic cells (DCs), independently of the TLR adaptor MYD88, but through the receptors macrophage-inducible C-type lectin or macrophage C-type lectin,[82] demonstrating that *H. pylori* metabolites also activate innate responses.

In contrast, several studies have shown that the T4SS has no impact or only a minor effect on activation of a proinflammatory response in myeloid cells.[77,83,84] The effect of CagA on macrophages has been extensively studied. Odenbreit et al demonstrated that CagA is cleaved and phosphorylated in human and murine macrophage cell lines infected with *H. pylori* strain P12.[85] However, the phosphorylated form of the full length CagA was not detected in this study, and a marked reduction of the cleaved phosphorylated CagA was observed when cells were infected with a *cagE* mutant,[85] suggesting that the T4SS is also involved in translocation of CagA into macrophages. In contrast, using the strain 60190, our group evidenced that full length CagA is phosphorylated in RAW 264.7 murine macrophages and when cells are infected with a *cagE* mutant, CagA is found in the cytoplasm of macrophages and phosphorylated,[86] indicating that phagocytosis is the main event leading to CagA phosphorylation; supporting this postulate, CagA phosphorylation is markedly reduced in macrophages infected with strain 7.13, which adheres to, but is not phagocytized by macrophages.[86] Notably, full length CagA is also phosphorylated in DCs,[87] and CagA can be delivered into DCs by outer membrane vesicles.[88]

Further, pCagA stimulates induction of heme oxygenase-1 in macrophages[86] and DCs.[88] In macrophages, heme oxygenase-1 dampens the proinflammatory/antibacterial M1 response and increases the anti-inflammatory Mreg phenotype.[86] Interestingly, C57BL/6 mice infected with a *cagA*-deficient strain are less colonized, but expresses more transcripts encoding for TNF-α, IL-6, IL-12B, and IL-1β in gastric tissues compared to animals infected with the parental strain.[89] The authors found that CagA in macrophages interacts with SHP1, thus inhibiting polyubiquitination and signaling of TRAF6.[89] Similarly, DCs infected with *H. pylori* D*cagA* produce more TNF-α and IL-12p40, but less IL-10, than the WT strain.[87,90] Increased CagA-dependent IL-10 production stimulates STAT3 phosphorylation/activation, thus impairing DC function.[90] Lastly, induction of SOCS3 in DCs by *H. pylori* through a mechanism that requires the T4SS reduces cytokine release and reduces T-cell proliferation.[91] Thus, contrary to GECs, CagA suppresses the innate response in myeloid cells (Figure 1). The impact of these observations on immunopathogenesis remains to be determined, but we propose that CagA supports immune escape of *H. pylori*.

Regulation of the Immune Response by the Gastric Microbiota

Germ-free (GF) INS-GAS mice exhibit a marked delay in development of gastritis and neoplasia compared with specific-pathogen-free animals.[92] Similarly, pretreatment of C57BL/6 mice with antibiotics results in less severe *H. pylori*-induced inflammation than untreated mice.[93] These data indicate that the trialogue between the host, the microbiota, and the pathogen plays an essential role in regulation of gastric disease.

Transgenic expression of the *cagA* gene in the adult midgut epithelium of *Drosophila* melanogaster leads to proliferation of stem cells, but not enterocytes, and the innate induction of the antimicrobial peptide diptericin and the enzyme dual oxidase.[94] In addition, the *Drosophila* gut is largely colonized by the bacterium *Acetobacter pasteurianus*, whereas *A. pasteurianus* and *Lactobacillus brevis* are both dominant in CagA-transgenic flies. Notably, (1) the microbiome is not altered in transgenic *Drosophila* expressing diptericin, and (2) the CagA-dependent overexpression of diptericin and dual oxidase is not observed in GF flies,[94] indicating that regulation of the innate response is a consequence of the perturbation of the gut microbiota. These effects are not observed in flies expressing a non-phosphorylatable CagA,[94] demonstrating that induction of the innate response through the *Drosophila* microbiota depends on CagA phosphorylation. Although colonization of GF *Drosophila* with *A. pasteurianus* and *L. brevis*, but not monoassociation with *A. pasteurianus* or *L. brevis*, resulted in increased stem cell proliferation, the authors did not investigate intestinal innate responses under these conditions.

In mice, inconsistent results have been published regarding the effect of *H. pylori* infection on the gastric microbiota. Differences may be due to the models of infection, the source of the mouse colonies, and the various methods used to analyze the microbiome. Highlighting these discrepancies, Tan et al did not observe significant changes in the gastric microbiome of conventional C57BL/6 mice infected with *H. pylori* using standard culture methods and terminal-restriction fragment length polymorphism analysis.[95] In contrast, 16S rDNA sequencing has revealed that *H. pylori* infection is associated with: (1) increased abundance of Firmicutes and a reduction in Bacteroidetes in INS-GAS mice[92]; (2) a prevalence of Proteobacteria, including the families *Pasteurellaceae*, *Erysipelotrichaceae*, and

Halomonadaceae, in specific-pathogen-free C57BL/6J mice[96]; and (3) a reduced abundance of Bacteroidetes and Firmicutes in C57BL/6 animals.[97] Thus, it is likely that perturbations in the gastric microbiota may influence *H. pylori*-induced immune response. Treatment of mice with antibiotics results in an increase of cluster IV and XIVa *Clostridium* spp. and a reduction of recruitment of $CD4^+$ cells and *Ifng* mRNA expression in the gastric mucosa.[93] Interestingly, Ge et al have observed that the abundance of Bacteroidetes is higher and Firmicutes lower in the stomach of C57BL/6 mice from the Jackson Laboratory (Jax) compared with animals from Taconic Bioscience (Tac).[98] In addition, the phyla Deferribacteres and Verrucomicrobia are only present in Tac mice.[98] Moreover, the microbial community of the stomach of Jax mice, but not Tac mice, was significantly altered by *H. pylori* infection[98] Concomitantly, the *H. pylori*-induced expression of *Il1b*, *Il17a*, and *Reg3g* was higher in Jax vs Tac mice.[98] In addition to showing that experimental *H. pylori* infection can be influenced by the source of the animals, these results also indicate that the microbial community of the stomach is associated with differences in immune response.

Although the human gastric microbiome has been analyzed in different populations around the world, a consensus has been developed regarding its composition. Most of the gastric microbiota in humans belongs to the phyla Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria, and the dominant genera are *Streptococcus*, *Prevotella*, *Neisseriae*, *Haemophilus*, and *Rothia*.[99–102] Overall, there is no difference between the antrum and corpus region.[100] However, the effect of *H. pylori* on the gastric microbiome is more controversial: although Bik et al have reported that there is no significant change in the composition of the gastric microbiota during *H. pylori* infection,[99] others have reported a reduction of the relative abundance of Proteobacteria and an increase of the *Streptococcus* genus in infected Chinese patients[100] as well as higher abundance of Proteobacteria and Acidobacteria in Amerindian subjects.[103] Further, in the Colombian Andes, the inhabitants of the town of Túquerres have a 25-fold higher risk of GC than inhabitants of the coastal town of Tumaco, despite similarly *H. pylori* infection rates; the gastric microbiota of the *H. pylori*-infected high-risk patients is dominated by the Fusobacteria *Leptotrichia wadei* and the genus *Veillonella*, whereas the genus *Staphylococcus*, *Neisseria*, *Porphyromonadaceae*, and *Flavobacterium* are more abundant in infected individuals from the low-risk region.[102] The effect of these microbial changes on *H. pylori*-induced mucosal immune response deserves further investigation. In a Chilean population, *H. pylori* infection in children is associated with a more diverse gastric microbiota, including a reduced abundance of Actinobacteria and an increase of the genera *Streptococcus, Actinomyces, Granulicatella*, or *Rothia*, compared with uninfected patients, whereas the microbiome of the adults is not modified by *H. pylori* infection.[104] In parallel, the gastric expression of the genes encoding for TGF-β and for the Treg markers FOXP3 and IL-10 is enhanced in *H. pylori*-infected children compared with uninfected children and infected adults.[104] A similar study performed in China also revealed that *H. pylori*-positive children exhibit reduced relative abundance of Actinobacteria compared with uninfected patients and that *FOXP3* mRNA levels are positively correlated with *TGFB1* and *IL10* levels as well as *H. pylori* abundance.[105] This suggests that the composition of the gastric microbiota is involved in downregulation of a specific pro-inflammatory response by supporting Treg function and is thus critical for immune tolerance and the persistence of the pathogen. Unfortunately, expression of other innate and T cell lineage markers has not been investigated in these studies.

Genetic Polymorphisms

In humans, multiple polymorphisms in genes encoding for immune effectors have been shown to influence gastric innate responses and consequently the outcome of *H. pylori* infection (Table 1).

NOD

The homozygous *NOD1*+796G>A variation is associated with higher gastritis scores and increased levels of *CXCL8* and *COX2* mRNA levels in *H. pylori*-infected patients from Korea, notably in patients infected with bacteria carrying an intact *cag*PAI.[106] Recently, the same conclusion has been drawn from a study in Chile: among 4 polymorphisms in the *NOD1* gene and 6 in *NOD2*, only the *NOD1*+796G>A change is associated with intestinal-type, but not diffuse-type, GC among subjects infected with *cag*PAI^+ *H. pylori.*[107] Duodenal ulceration is also significantly associated with this homozygous variation in Hungarian individuals.[108]

NOD2 variants are not associated with gastritis levels or gastric ulcer development.[107,109] However, the *NOD2*+2104C>T polymorphism, which has been shown to be associated with susceptibility for development of Crohn's disease,[110] is associated with reduced NF-κB activation and significantly associated with gastric lymphoma in *H. pylori*-infected patients.[109] Carriers of the T allele have a more than doubled risk for developing lymphoma than controls.[109]

Thus, the reduced ability of host cells, notably GECs, to sense bacteria intracellularly is linked with increased inflammation and GC risk.

TLR4

TLR4 belongs to a family of pattern recognition receptors that recognizes lipopolysaccharide (LPS). *TLR4*+896A>G results in replacement of a conserved aspartic acid residue with glycine at amino acid 299 and alteration in the response to LPS challenge; *TLR4*+1196C>T yields a threonine to isoleucine exchange at position 399. But others exist, including the *TLR4*+3725G>C, leading to faster transcript degradation, and *TLR4*+2856T>C.

Published data regarding the role of *TLR4* polymorphisms are controversial and seem dependent on ethnicity. In Caucasian individuals from Scotland, Poland, and the United States, the *TLR4* polymorphism is not associated with risk of *H. pylori* infection, but the *TLR4*+896G

Table 1. Role of the Polymorphisms for the Genes Encoding for Innate Immune Effectors on *H. pylori*-mediated Diseases

Gene	Polymorphism	Population	Effects	References
NOD1	+796G>A	Korean	↑ Gastritis	108
		Chilean	↑ GC	109
		Hungarian	↑ DU	110
NOD2	+2104C>T	German	↑ Gastric lymphoma	111
TLR4	+896A>G	Caucasian	↑ Gastritis, ↑ GC	113
		Mexican	↑ DU, ↑ GC	115
		Indian	↑ Gastritis	118
		Iranian	↑ Gastritis	119
	+1196C>T	Mexican	↑ DU, ↑ GC	115
		Indian	↑ Gastritis, ↑ GC	118
	+2856T>C	Chinese	↓ IM, ↓ GC	121
	+3725G>C	Japanese	↑ AG	122
TLR9	−1237T>C	Scottish	↑ AG	125
		Brazilian	↑ GC	127
	+2848G>A	Indian Tamils	↑ Infection	128
		Mexican	↑ DU	129
CXCL8	−251T>A	Japanese	↑ Gastritis, ↑ AG, ↑ GC	130
		Chinese	↑ Gastritis, ↑ AG, ↑ GC	131
		Korean	↑ AG, ↑ GC	133
		Korean	↑ Gastritis, ↑ AG, ↑ GC	134
		Caucasian	↑ Gastritis	135
		East European	↑ DU	110
		Mexican	↑ GC	117
		Chinese	↑ GC	136
	−845T>C	Brazilian	↑ Gastritis, ↑ GC	137
IL1A	−899C>T	Jamaican	↓ Infection	139
IL1B	−31C>T	Caucasian	↑ GC	142, 143
		Italian	↑ IM	147
	−511C>T	Caucasian	↑ GC	142, 143
		Portuguese	↑ GC	144
		Chinese	↑ GC	146
IL1RN	IL1RN*2	Caucasian	↑ GC	142, 143
		Portuguese	↑ GC	144
		Italian	↑ IM	147
		German	↑ Gastritis, ↑ AG, ↑ IM	138
TNF	−308G>A	Korean	↑ Infection	149
		Italy	↑ Infection	147
		Korean	↑ GC	150
		US Caucasian	↑ GC	143
		Portuguese	↑ GC	151
		Chinese	↑ GC	152
IL10	−1082A/−819T/−592A	US Caucasian	↑ GC	143
		Chinese	↓ GC	152
		Taiwanese	↓ GC	154
		Japanese	↓ GC	155
		Mexican	↓ IM, ↓ GC	156
		Venezuelan	↓ IM, ↓ GC	157

AG, Atrophic gastritis, DU, duodenal ulcer; GC, gastric cancer; IM, intestinal metaplasia.

carriers have more severe histologic gastritis, hypochlorhydria, and increased risk for GC.[111] However, GC is not related to the *TLR4*+3725G>C polymorphism in individuals from Germany, Lithuania, and Latvia.[112]

In patients from Mexico, the expression of *CCL2*, *CCL3*, *TNF*, and *IL10* is significantly higher in patients harboring at least one of the *TLR4*+896A>G or *TLR4*+1196C>T variants.[113] In contrast, expression of the genes encoding for innate cytokines IL-1β and IL-6, and chemokines IL-8 and GROα, is lower in patients with the single nucleotide polymorphisms.[113] But overall, the *TLR4*+896A>G and/or *TLR4*+1196C>T variants are more frequent among infected patients with duodenal ulcer and GC than in patients with gastritis.[113] In contrast, other studies have shown no correlation between *TLR4*+896A>G and IM/GC and/or duodenal ulceration in Peruvian, Mexican, and Hungarian populations.[108,114,115]

In an Indian population, the *TLR4*+1196T allele is associated with risk for severe gastritis development and formation of premalignant lesions, and the *TLR4*+896G allele is associated with enhanced neutrophil infiltration, independently of CagA seropositivity.[116] However, this last variant is not associated with risk for GC.[116]

In a cohort of 436 Iranians, the *TLR4*+896G allele frequency is associated with increased mononuclear cell infiltration and risk for the development of chronic gastritis upon *H. pylori* infection.[117] The same group has observed that expression of the genes encoding for IL-6, IL-23, and TGF-β1, which are required for Th17 differentiation, as well as for the Th17 cytokines IL-17, IL-21, and IL-23, is significantly higher in infected patients with the *TLR4*+896A>G polymorphism.[118]

The *TLR4*+896A>G and/or *TLR4*+1196C>T variants are not associated with IM and GC in patients from China,[119] and they do not exist in the Japanese population.[120] However, Chinese persons harboring the *TLR4*+2856T>C genotype exhibit less IM and gastric neoplasia than control patients,[119] and the *TLR4*+3725G>C polymorphism is associated with increased risk of severe gastric atrophy in Japanese persons.[120]

Overall, the loss of TLR4 functionality is associated with increased inflammation and risk for gastric neoplasia. This result seems paradoxical, because LPS sensing is linked to a pro-inflammatory response and *H. pylori* LPS has low potency relative to *E. coli* LPS. However, it is possible that LPS might stimulate immune cells during long-term infection. Moreover, it has been proposed that the failure to recognize infection by appropriate receptors may lead to an imbalance of pro- and anti-inflammatory mediators and that loss of TLR4 function reduces IL-10 production and the control of inflammation.[121] Notably, *TLR4* polymorphisms are associated with exacerbated inflammation in patients with Crohn's disease.[122]

TLR9

The *TLR9*–1237T>C promoter polymorphisms creates a potential NF-κB binding site and thus increases the transcription of *TLR9*.[123] Similarly, the *TLR9*+2848G>A polymorphism is associated with increased *TLR9* expression.

In a Caucasian population from Scotland, the C allele at –1237 is not associated with *H. pylori* infection, but is linked to development of gastric atrophy,[123] though not with GC.[124] In contrast, this polymorphism is associated with a higher risk of gastric carcinogenesis in patients from Brazil.[125] The A allele at position 2848 is associated with long-term infection by *H. pylori* in Indian Tamils[126] and is more frequent in Mexican patients with duodenal ulcer than in the group with non-atrophic gastritis.[127]

In gastric biopsies, the expression of genes encoding for IL-1β and TNF-α is lower in patients with −1237C or 2848A *TLR9* variant alleles.[127] The authors of this study proposed that these mutations have modified the recognition of the ligand and thus the signaling pathways leading to cytokine induction.[127] Phenotypically, the impaired production of IL-1β, recognized as a strong inhibitor of acid production, would result in increased acid secretion and increased risk for duodenal ulcer.

CXCL8

The gene *CXCL8* encodes for IL-8 and may exhibit *CXCL8*–251T>A or *CXCL8*–845T>C polymorphisms in the promoter region.

In *H. pylori*-infected Japanese, Chinese, and Korean patients, the A/A variant in position –251, which leads to increased *CXCL8* promoter activity,[128–131] is associated with higher gastric neutrophil infiltration, atrophy, IM,[129,130] and GC compared with the A/T or T/T genotypes.[128,129,132] The same A/A genotype in European Caucasian patients is significantly increased in patients with gastritis, but is not correlated with GC.[133] In another study from South Korea, the T/A and A/A genotypes correlated with increased IL-8 in the gastric mucosa of *H. pylori*-infected patients compared to the T/T genotype, and the A allele significantly increased the risk of severe atrophic gastritis and GC.[131] The A/T genotype correlates with duodenal ulcer in *H. pylori*-infected subjects from Eastern Europe[108] and with GC in patients from Mexico.[115] Intriguingly, the *CXCL8*–251T/T genotype has been correlated with higher transcriptional activity of *CXCL8* and increased risk of GC in a population of Chinese Veterans infected with *H. pylori*.[134]

In contrast, in a Brazilian cohort, the *CXCL8*–845T>C polymorphism, but not the *CXCL8*–251A>T, has been correlated with increased risk of developing chronic gastritis and GC in *H. pylori*-infected individuals.[135] The authors also showed that the C variant in position –845 is responsible for the presence of binding sites for the transcription factors NF-κB and CREB1.[135]

Together, these data indicate that polymorphisms in the promoter region of the *CXCL8* gene are associated with increased IL-8 synthesis, inflammation, and risk for neoplastic transformation.

IL1

The *IL1* cluster on chromosome 2q contains 3 genes, *IL1A*, *IL1B*, and *IL1RN*, which encode the pro-inflammatory cytokines IL-1α and IL-1β, and the IL-1 receptor antagonist, respectively. The *IL1A*–899C>T polymorphism has been

described in the promoter region and the T allele yields increased IL-1α production. Three diallelic polymorphisms in *IL1B* have been reported, all representing C>T transitions at positions –511, –31, and +3954, and overall are associated with increased IL-1β synthesis. *IL1RN* contains a variable number of tandem repeats of 86 bp in intron 2, with *IL1RN**1 representing 4 repeats, *IL1RN**2 having 2 repeats, and a less common *IL1RN**3 containing 5 repeats; *IL1RN**2 is associated with higher production of IL-1β.[136]

Jamaican children with the *IL1A*–889T allele exhibit a lower risk of *H. pylori* positivity,[137] suggesting that IL-1α might control the infection. However, this polymorphism is not associated with disease outcome.[138,139]

In Caucasian populations from Scotland and Poland, *H. pylori*-infected patients with low acid secretion exhibit higher frequency of the haplotype *IL1B*–31T/*1L-1B*–511T and/or homozygosity for *IL1RN**2.[140] However, it has been noted that near-complete linkage disequilibrium exists between polymorphisms at −31 and −511.[140] Carriers of the T allele and homozygotes for *IL1RN**2 are also at higher risk for GC development.[140] These findings have been recapitulated in patients from the United States.[141] Importantly, it has been confirmed that the –31T allele is associated with increased IL-1β promoter activity.[140]

IL1B–511C/T heterozygotes from Portugal display a significantly increased GC risk;[142] but in this study, the number of individuals with T/T genotype was too small to determine a risk estimate. In addition, a significant association was observed between *IL1RN**2 genotype and the risk of intestinal-type GC.[142] The increased risk of intestinal GC conferred by *IL1B*–511C>T is enhanced in individuals homozygous for the *IL1RN**2 allele,[142] and further enhanced in those infected with *vacA*s1$^+$, *vacA*m1$^+$, or *cagA*$^+$ strains.[143] A similar association between the *IL1B*−511T/T genotype and the presence of *H. pylori* has been reported to increase the risk of GC in a Chinese population.[144] Further, Zambon et al have shown that the presence of *cagA* or *oipA* and *IL1B*–31C>T or *IL1RN**2 is associated with IM.[145]

Critically, Hwang et al have also reported in a Japanese cohort that carriers of the T/T genotype have increased IL-1β concentration and infiltration of neutrophils and mononuclear cells in the gastric mucosa compared with T/C and C/C genotypes at *IL1B-511*.[146] Similarly, the *IL1RN**2 allele is correlated with a high concentration of mucosal IL-1b and infiltration of myeloid cells.[146] A synergistic effect between the 2 loci was evidenced by these authors.[146] In a German cohort, the presence of *IL1RN*2* has been associated with increased *IL1B* mRNA expression in gastric tissues, more severe inflammation, and increased prevalence of IM and atrophic gastritis[136]; surprisingly, the *IL1B–511T* allele is not correlated with increased *IL1B* gene expression, but still with disease progression.[136]

In conclusion, polymorphisms in the *IL1* cluster that enhance IL-1β production support gastritis and GC.

TNF

TNF-α is partly regulated at the transcriptional level, and studies have implicated polymorphisms in the promoter region as potential determinants of disease susceptibility. The best-studied mutations are 3 G to A transitions at positions −308 and −238 in the *TNF* gene. The −308A and −238A alleles have been correlated with increased and reduced TNF-α production, respectively.

In a Korean population, the *TNF*−308G>A polymorphism, but not *TNF*−238G>A, is related to an increased risk of infection with *H. pylori*.[147] Similar observations have been made in Italy.[145] Moreover, in Korean patients,[148] in a multicenter case-control study conducted in the United States,[141] in a cohort of patients from Portugal,[149] or in a Chinese study,[150] the risk of GC is more pronounced for *TNF*−308A/A homozygotes compared with heterozygotes. Concomitantly, the proportion of individuals carrying the *TNF*–238A allele is significantly lower in the GC group.

IL10

Three single nucleotide polymorphisms, located in the promoter region at positions −1082 (G>A), −819 (C>T), and −592 (C>A), have been identified in the *IL10* gene and lead to a reduction of IL-10 synthesis.

Rad et al have confirmed that *H. pylori*-infected patients from Germany harboring *IL10*−1082A/A or *IL10*−592A/A exhibit less *IL10* mRNA transcripts in the gastric mucosa than those with the *IL10*−1082A(G)/G or *IL10*−592A(C)/C genotypes, respectively.[136] Further, *IL10* mRNA expression is lower in carriers of the *IL-10*−1082A/−819T/−592A haplotype compared with the GCC carriers.[136] Additionally, the prevalence of *cagA*$^+$, *vacAs1*$^+$, and *babA2*$^+$ strains is higher among GCC carriers than among ATA carriers, but there is no significant correlation between *IL10* polymorphisms and the degree of gastritis or IM development.[136] A second study performed in Germany showed no association between *IL10* polymorphisms and gastric ulceration.[151]

However, El-Omar et al found that United States Caucasian patients harboring the low IL-10-producer −1082A/−819T/−592A haplotype have significantly increased risk of GC.[141] Contrasting results have been obtained for other populations: The high IL-10-producer haplotype −1082G/−819C/−592C is more present in GC than the low IL-10 haplotype (ATA) in Taiwanese[152] and Japanese patients.[153] Similarly, in China, an elevated risk of GC is observed in subjects with *H. pylori* infection and *IL10*–1082G carriers.[150] In Mexico and Venezuela, the *IL10*–592C/C and *IL10*–1082G/G genotypes increase risk of IM, dysplasia, and GC.[154,155] The difference between United States and Asian patients can be explained by the finding that the allele frequency of the *IL10*–1082 polymorphism is markedly different among Caucasians and East Asians.[156] Nonetheless, *IL10* polymorphisms influence *H. pylori*-induced inflammation and severity of disease.

Multi-polymorphisms, or Not

The study of the effect of polymorphisms in the genes encoding for the immune response on *H. pylori*-induced inflammation and disease has led to contradictory results in different cohorts. Multiple synergistic and opposite polymorphisms may explain certain discrepancies. For example,

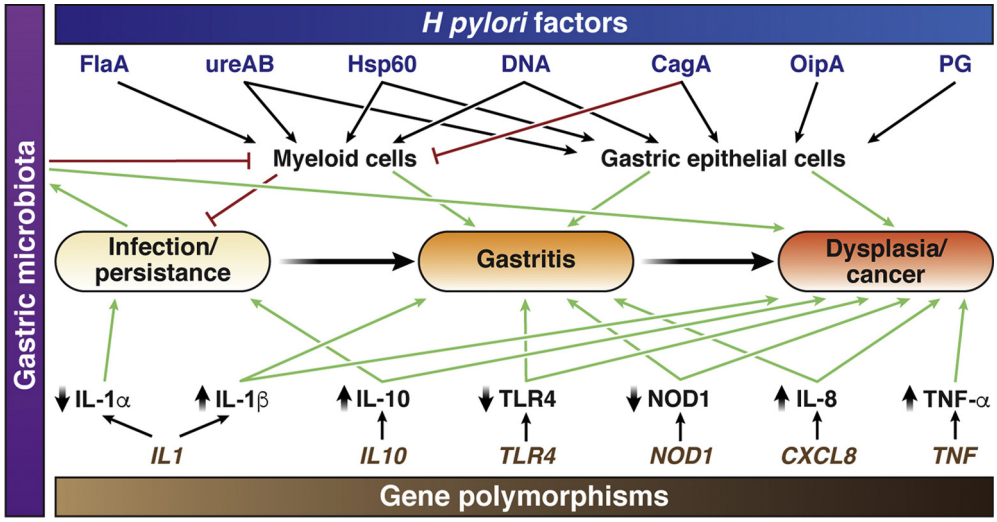

Figure 2. A model for the role of the innate response in disease progression. Illustration of the impact of *H. pylori* factors, the gastric microbiota, and genetic susceptibility on the different stages of *H. pylori*-induced diseases.

the combination of the carriage of *IL1B*−511T, *IL1RN**2 homozygosity (high IL-1β), and *TNF*−308G (high TNF-α) defines a high-risk genetic profile for both chronic atrophic gastritis and GC.[141,149] Homozygosity for the low IL-10 haplotype ATA further increases GC risk.[141] In Korean patients, the *IL10*–592A/A genotype (low IL-10) and the *CXCL8*–251A/A genotype (high IL-8) each are associated with a greater relative risk of developing GC, and the combination yielded a synergistic increase in risk.[157] Such findings suggest that a combination of multiple host immune factors is involved in inflammation and disease progression. Nonetheless, the effect of these inflammatory polymorphisms must be taken with caution. However, a study performed in cohort of 950 Irish patients showed no correlation between 11 variants alleles in the *TLR4*, *IL1* cluster, *TNF*, and *IL10* genes and the development of chronic atrophic gastritis or IM.[158]

Concluding Remarks

H. pylori possesses an arsenal to induce the innate response of GECs and immune cells, leading to inflammation and chronic disorders. However, the bacterium has also developed strategies to dampen inflammation and escape the immune response. The gastric microbiota composition is affected by the infection; this dysbiosis dampens the innate response and favors neoplasia. Inflammation and disease progression are also regulated by polymorphisms of the genes encoding for immune effectors. These conclusions are summarized in Figure 2.

The co-evolution between *H. pylori* and its host persists and is now molded by the modern era through population movements, improved hygiene, changes in diet, and use of drugs, notably antibiotics. In addition, the decreased prevalence of *H. pylori*, particularly in industrialized countries, has been linked to the rise of esophageal diseases, metabolic diseases, and allergic disorders. Continuing to understand the regulation of the gastric innate response in this context requires our full attention.

References

1. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY, Chan FKL, Sung JJY, Kaplan GG, Ng SC. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology 2017;153:420–429.
2. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process–First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 1992;52:6735–6740.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–249.
4. Moodley Y, Linz B, Bond RP, Nieuwoudt M, Soodyall H, Schlebusch CM, Bernhoft S, Hale J, Suerbaum S, Mugisha L, van der Merwe SW, Achtman M. Age of the association between *Helicobacter pylori* and man. PLoS Pathog 2012;8:e1002693.
5. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci U S A 1996;93:14648–14653.
6. Segal ED, Lange C, Covacci A, Tompkins LS, Falkow S. Induction of host signal transduction pathways by *Helicobacter pylori*. Proc Natl Acad Sci U S A 1997;94:7595–7599.
7. Odenbreit S, Puls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science 2000;287:1497–1500.
8. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, Konig W, Backert S. Helicobacter exploits integrin for type IV secretion and kinase activation. Nature 2007;449:862–866.

9. Selbach M, Moese S, Hauck CR, Meyer TF, Backert S. Src is the kinase of the *Helicobacter pylori* CagA protein in vitro and in vivo. J Biol Chem 2002;277:6775–6778.
10. Mueller D, Tegtmeyer N, Brandt S, Yamaoka Y, De Poire E, Sgouras D, Wessler S, Torres J, Smolka A, Backert S. c-Src and c-Abl kinases control hierarchic phosphorylation and function of the CagA effector protein in Western and East Asian *Helicobacter pylori* strains. J Clin Invest 2012;122:1553–1566.
11. Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295:683–686.
12. Backert S, Moese S, Selbach M, Brinkmann V, Meyer TF. Phosphorylation of tyrosine 972 of the *Helicobacter pylori* CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. Mol Microbiol 2001; 42:631–644.
13. Higashi H, Nakaya A, Tsutsumi R, Yokoyama K, Fujii Y, Ishikawa S, Higuchi M, Takahashi A, Kurashima Y, Teishikata Y, Tanaka S, Azuma T, Hatakeyama M. *Helicobacter pylori* CagA induces Ras-independent morphogenetic response through SHP-2 recruitment and activation. J Biol Chem 2004;279:17205–17216.
14. Oldani A, Cormont M, Hofman V, Chiozzi V, Oregioni O, Canonici A, Sciullo A, Sommi P, Fabbri A, Ricci V, Boquet P. *Helicobacter pylori* counteracts the apoptotic action of its VacA toxin by injecting the CagA protein into gastric epithelial cells. PLoS Pathog 2009;5:e1000603.
15. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995; 55:2111–2115.
16. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. Gut 1997; 40:297–301.
17. Crabtree JE, Farmery SM, Lindley IJ, Figura N, Peichl P, Tompkins DS. CagA/cytotoxic strains of *Helicobacter pylori* and interleukin-8 in gastric epithelial cell lines. J Clin Pathol 1994;47:945–950.
18. Sharma SA, Tummuru MK, Miller GG, Blaser MJ. Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. Infect Immun 1995; 63:1681–1687.
19. Sharma SA, Tummuru MK, Blaser MJ, Kerr LD. Activation of IL-8 gene expression by *Helicobacter pylori* is regulated by transcription factor nuclear factor-kappa B in gastric epithelial cells. J Immunol 1998; 160:2401–2407.
20. Argent RH, Kidd M, Owen RJ, Thomas RJ, Limb MC, Atherton JC. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of *Helicobacter pylori*. Gastroenterology 2004; 127:514–523.
21. Cox JM, Clayton CL, Tomita T, Wallace DM, Robinson PA, Crabtree JE. cDNA array analysis of cag pathogenicity island-associated *Helicobacter pylori* epithelial cell response genes. Infect Immun 2001; 69:6970–6980.
22. Keates S, Keates AC, Warny M, Peek RM Jr, Murray PG, Kelly CP. Differential activation of mitogen-activated protein kinases in AGS gastric epithelial cells by *cag*+ and *cag*- *Helicobacter pylori*. J Immunol 1999; 163:5552–5559.
23. Tummuru MK, Sharma SA, Blaser MJ. *Helicobacter pylori picB*, a homologue of the *Bordetella pertussis* toxin secretion protein, is required for induction of IL-8 in gastric epithelial cells. Mol Microbiol 1995;18:867–876.
24. Nagy TA, Allen SS, Wroblewski LE, Flaherty DK, Slaughter JC, Perez-Perez G, Israel DA, Peek RM Jr. *Helicobacter pylori* induction of eosinophil migration is mediated by the *cag* pathogenicity island via microbial-epithelial interactions. Am J Pathol 2011;178:1448–1452.
25. Ogura K, Takahashi M, Maeda S, Ikenoue T, Kanai F, Yoshida H, Shiratori Y, Mori K, Mafune KI, Omata M. Interleukin-8 production in primary cultures of human gastric epithelial cells induced by *Helicobacter pylori*. Dig Dis Sci 1998;43:2738–2743.
26. Selbach M, Moese S, Meyer TF, Backert S. Functional analysis of the *Helicobacter pylori cag* pathogenicity island reveals both VirD4-CagA-dependent and VirD4-CagA-independent mechanisms. Infect Immun 2002; 70:665–671.
27. Lin AS, Dooyema SDR, Frick-Cheng AE, Harvey ML, Suarez G, Loh JT, McDonald WH, McClain MS, Peek RM Jr, Cover TL. Bacterial energetic requirements for *Helicobacter pylori* Cag Type IV secretion system-dependent alterations in gastric epithelial cells. Infect Immun 2020;88:e00790–19.
28. Keates S, Sougioultzis S, Keates AC, Zhao D, Peek RM Jr, Shaw LM, Kelly CP. *cag*+ *Helicobacter pylori* induce transactivation of the epidermal growth factor receptor in AGS gastric epithelial cells. J Biol Chem 2001;276:48127–48134.
29. Kim SY, Lee YC, Kim HK, Blaser MJ. *Helicobacter pylori* CagA transfection of gastric epithelial cells induces interleukin-8. Cell Microbiol 2006;8:97–106.
30. Gobert AP, Verriere T, de Sablet T, Peek RM Jr, Chaturvedi R, Wilson KT. Haem oxygenase-1 inhibits phosphorylation of the *Helicobacter pylori* oncoprotein CagA in gastric epithelial cells. Cell Microbiol 2013; 15:145–156.
31. 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.
32. Xu S, Wu X, Zhang X, Chen C, Chen H, She F. CagA orchestrates eEF1A1 and PKCdelta to induce interleukin-6 expression in *Helicobacter pylori*-infected gastric epithelial cells. Gut Pathog 2020;12:31.
33. Crabtree JE, Xiang Z, Lindley IJ, Tompkins DS, Rappuoli R, Covacci A. Induction of interleukin-8 secretion from gastric epithelial cells by a cagA negative isogenic mutant of *Helicobacter pylori*. J Clin Pathol 1995;48:967–969.
34. Brandt S, Kwok T, Hartig R, Konig W, Backert S. NF-kappaB activation and potentiation of proinflammatory

responses by the *Helicobacter pylori* CagA protein. Proc Natl Acad Sci U S A 2005;102:9300–9305.
35. Argent RH, Hale JL, El-Omar EM, Atherton JC. Differences in *Helicobacter pylori* CagA tyrosine phosphorylation motif patterns between western and East Asian strains, and influences on interleukin-8 secretion. J Med Microbiol 2008;57:1062–1067.
36. Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, Nagai S, Koyasu S, Gilman RH, Kersulyte D, Berg DE, Sasakawa C. *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. Cell Host Microbe 2009;5:23–34.
37. Lee IO, Kim JH, Choi YJ, Pillinger MH, Kim SY, Blaser MJ, Lee YC. *Helicobacter pylori* CagA phosphorylation status determines the gp130-activated SHP2/ERK and JAK/STAT signal transduction pathways in gastric epithelial cells. J Biol Chem 2010; 285:16042–16050.
38. Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Memet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori cag* pathogenicity island. Nat Immunol 2004;5:1166–1174.
39. Suarez G, Romero-Gallo J, Piazuelo MB, Wang G, Maier RJ, Forsberg LS, Azadi P, Gomez MA, Correa P, Peek RM Jr. Modification of *Helicobacter pylori* peptidoglycan enhances NOD1 activation and promotes cancer of the stomach. Cancer Res 2015;75:1749–1759.
40. Allison CC, Kufer TA, Kremmer E, Kaparakis M, Ferrero RL. *Helicobacter pylori* induces MAPK phosphorylation and AP-1 activation via a NOD1-dependent mechanism. J Immunol 2009;183:8099–8109.
41. Boughan PK, Argent RH, Body-Malapel M, Park JH, Ewings KE, Bowie AG, Ong SJ, Cook SJ, Sorensen OE, Manzo BA, Inohara N, Klein NJ, Nunez G, Atherton JC, Bajaj-Elliott M. Nucleotide-binding oligomerization domain-1 and epidermal growth factor receptor: critical regulators of beta-defensins during *Helicobacter pylori* infection. J Biol Chem 2006;281:11637–11648.
42. Tran LS, Tran D, De Paoli A, D'Costa K, Creed SJ, Ng GZ, Le L, Sutton P, Silke J, Nachbur U, Ferrero RL. NOD1 is required for *Helicobacter pylori* induction of IL-33 responses in gastric epithelial cells. Cell Microbiol 2018;20:e12826.
43. Kaparakis M, Turnbull L, Carneiro L, Firth S, Coleman HA, Parkington HC, Le Bourhis L, Karrar A, Viala J, Mak J, Hutton ML, Davies JK, Crack PJ, Hertzog PJ, Philpott DJ, Girardin SE, Whitchurch CB, Ferrero RL. Bacterial membrane vesicles deliver peptidoglycan to NOD1 in epithelial cells. Cell Microbiol 2010; 12:372–385.
44. Irving AT, Mimuro H, Kufer TA, Lo C, Wheeler R, Turner LJ, Thomas BJ, Malosse C, Gantier MP, Casillas LN, Votta BJ, Bertin J, Boneca IG, Sasakawa C, Philpott DJ, Ferrero RL, Kaparakis-Liaskos M. The immune receptor NOD1 and kinase RIP2 interact with bacterial peptidoglycan on early endosomes to promote autophagy and inflammatory signaling. Cell Host Microbe 2014;15:623–635.
45. Suarez G, Romero-Gallo J, Piazuelo MB, Sierra JC, Delgado AG, Washington MK, Shah SC, Wilson KT, Peek RM Jr. Nod1 imprints inflammatory and carcinogenic responses toward the gastric pathogen *Helicobacter pylori*. Cancer Res 2019;79:1600–1611.
46. Varga MG, Shaffer CL, Sierra JC, Suarez G, Piazuelo MB, Whitaker ME, Romero-Gallo J, Krishna US, Delgado A, Gomez MA, Good JA, Almqvist F, Skaar EP, Correa P, Wilson KT, Hadjifrangiskou M, Peek RM Jr. Pathogenic *Helicobacter pylori* strains translocate DNA and activate TLR9 via the cancer-associated *cag* type IV secretion system. Oncogene 2016;35:6262–6269.
47. Schmausser B, Andrulis M, Endrich S, Lee SK, Josenhans C, Muller-Hermelink HK, Eck M. Expression and subcellular distribution of toll-like receptors TLR4, TLR5 and TLR9 on the gastric epithelium in *Helicobacter pylori* infection. Clin Exp Immunol 2004;136:521–526.
48. Uotani T, Murakami K, Uchida T, Tanaka S, Nagashima H, Zeng XL, Akada J, Estes MK, Graham DY, Yamaoka Y. Changes of tight junction and interleukin-8 expression using a human gastroid monolayer model of *Helicobacter pylori* infection. Helicobacter 2019;24:e12583.
49. Huang J, O'Toole PW, Doig P, Trust TJ. Stimulation of interleukin-8 production in epithelial cell lines by *Helicobacter pylori*. Infect Immun 1995;63:1732–1738.
50. Argent RH, Thomas RJ, Letley DP, Rittig MG, Hardie KR, Atherton JC. Functional association between the *Helicobacter pylori* virulence factors VacA and CagA. J Med Microbiol 2008;57:145–150.
51. Kim IJ, Lee J, Oh SJ, Yoon MS, Jang SS, Holland RL, Reno ML, Hamad MN, Maeda T, Chung HJ, Chen J, Blanke SR. *Helicobacter pylori* infection modulates host cell metabolism through VacA-dependent inhibition of mTORC1. Cell Host Microbe 2018;23:583–593.e8.
52. Tanahashi T, Kita M, Kodama T, Yamaoka Y, Sawai N, Ohno T, Mitsufuji S, Wei YP, Kashima K, Imanishi J. Cytokine expression and production by purified *Helicobacter pylori* urease in human gastric epithelial cells. Infect Immun 2000;68:664–671.
53. Beswick EJ, Pinchuk IV, Minch K, Suarez G, Sierra JC, Yamaoka Y, Reyes VE. The *Helicobacter pylori* urease B subunit binds to CD74 on gastric epithelial cells and induces NF-kappaB activation and interleukin-8 production. Infect Immun 2006;74:1148–1155.
54. Vanet A, Labigne A. Evidence for specific secretion rather than autolysis in the release of some *Helicobacter pylori* proteins. Infect Immun 1998;66:1023–1027.
55. Marcus EA, Scott DR. Cell lysis is responsible for the appearance of extracellular urease in *Helicobacter pylori*. Helicobacter 2001;6:93–99.
56. Ohta-Tada U, Takagi A, Koga Y, Kamiya S, Miwa T. Flagellin gene diversity among *Helicobacter pylori* strains and IL-8 secretion from gastric epithelial cells. Scand J Gastroenterol 1997;32:455–459.
57. Gewirtz AT, Yu Y, Krishna US, Israel DA, Lyons SL, Peek RM Jr. *Helicobacter pylori* flagellin evades toll-like receptor 5-mediated innate immunity. J Infect Dis 2004;189:1914–1920.
58. Smith MF Jr, Mitchell A, Li G, Ding S, Fitzmaurice AM, Ryan K, Crowe S, Goldberg JB. Toll-like receptor (TLR) 2

and TLR5, but not TLR4, are required for *Helicobacter pylori*-induced NF-kappa B activation and chemokine expression by epithelial cells. J Biol Chem 2003; 278:32552–32560.
59. Yamaguchi H, Osaki T, Kurihara N, Kitajima M, Kai M, Takahashi M, Taguchi H, Kamiya S. Induction of secretion of interleukin-8 from human gastric epithelial cells by heat-shock protein 60 homologue of *Helicobacter pylori*. J Med Microbiol 1999;48:927–933.
60. Yamaguchi H, Osaki T, Kurihara N, Taguchi H, Kamiya S. Reactivity of monoclonal antibody to HSP60 homologue of *Helicobacter pylori* with human gastric epithelial cells and induction of IL-8 from these cells by purified *H. pylori* HSP60. J Gastroenterol 1999;34(Suppl 11):1–5.
61. Takenaka R, Yokota K, Ayada K, Mizuno M, Zhao Y, Fujinami Y, Lin SN, Toyokawa T, Okada H, Shiratori Y, Oguma K. *Helicobacter pylori* heat-shock protein 60 induces inflammatory responses through the Toll-like receptor-triggered pathway in cultured human gastric epithelial cells. Microbiology (Reading) 2004; 150:3913–3922.
62. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of *Helicobacter pylori*. Proc Natl Acad Sci U S A 2000; 97:7533–7538.
63. Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology 2002;123:414–424.
64. Yamaoka Y, Kita M, Kodama T, Imamura S, Ohno T, Sawai N, Ishimaru A, Imanishi J, Graham DY. *Helicobacter pylori* infection in mice: Role of outer membrane proteins in colonization and inflammation. Gastroenterology 2002;123:1992–2004.
65. Odenbreit S, Kavermann H, Puls J, Haas R. CagA tyrosine phosphorylation and interleukin-8 induction by *Helicobacter pylori* are independent from AlpAB, HopZ and Bab group outer membrane proteins. Int J Med Microbiol 2002;292:257–266.
66. Ando T, Peek RM Jr, Lee YC, Krishna U, Kusugami K, Blaser MJ. Host cell responses to genotypically similar *Helicobacter pylori* isolates from United States and Japan. Clin Diagn Lab Immunol 2002;9:167–175.
67. Tabassam FH, Graham DY, Yamaoka Y. *Helicobacter pylori* activate epidermal growth factor receptor- and phosphatidylinositol 3-OH kinase-dependent Akt and glycogen synthase kinase 3beta phosphorylation. Cell Microbiol 2009;11:70–82.
68. Yamaoka Y, Kudo T, Lu H, Casola A, Brasier AR, Graham DY. Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in *Helicobacter pylori* infection. Gastroenterology 2004; 126:1030–1043.
69. Kudo T, Lu H, Wu JY, Graham DY, Casola A, Yamaoka Y. Regulation of RANTES promoter activation in gastric epithelial cells infected with *Helicobacter pylori*. Infect Immun 2005;73:7602–7612.
70. Lu H, Wu JY, Kudo T, Ohno T, Graham DY, Yamaoka Y. Regulation of interleukin-6 promoter activation in gastric epithelial cells infected with *Helicobacter pylori*. Mol Biol Cell 2005;16:4954–4966.
71. Lu H, Wu JY, Beswick EJ, Ohno T, Odenbreit S, Haas R, Reyes VE, Kita M, Graham DY, Yamaoka Y. Functional and intracellular signaling differences associated with the *Helicobacter pylori* AlpAB adhesin from Western and East Asian strains. J Biol Chem 2007;282:6242–6254.
72. Oleastro M, Cordeiro R, Ferrand J, Nunes B, Lehours P, Carvalho-Oliveira I, Mendes AI, Penque D, Monteiro L, Megraud F, Menard A. Evaluation of the clinical significance of homB, a novel candidate marker of *Helicobacter pylori* strains associated with peptic ulcer disease. J Infect Dis 2008;198:1379–1387.
73. Kusugami K, Ando T, Ohsuga M, Imada A, Shinoda M, Konagaya T, Ina K, Kasuga N, Fukatsu A, Ichiyama S, Nada T, Ohta M. Mucosal chemokine activity in *Helicobacter pylori* infection. J Clin Gastroenterol 1997;25-(Suppl 1):S203–210.
74. Gobert AP, Mersey BD, Cheng Y, Blumberg DR, Newton JC, Wilson KT. Cutting edge: urease release by *Helicobacter pylori* stimulates macrophage inducible nitric oxide synthase. J Immunol 2002;168:6002–6006.
75. Gobert AP, Bambou JC, Werts C, Balloy V, Chignard M, Moran AP, Ferrero RL. *Helicobacter pylori* heat shock protein 60 mediates interleukin-6 production by macrophages via a toll-like receptor (TLR)-2-, TLR-4-, and myeloid differentiation factor 88-independent mechanism. J Biol Chem 2004;279:245–250.
76. Lin SN, Ayada K, Zhao Y, Yokota K, Takenaka R, Okada H, Kan R, Hayashi S, Mizuno M, Hirai Y, Fujinami Y, Oguma K. *Helicobacter pylori* heat-shock protein 60 induces production of the pro-inflammatory cytokine IL8 in monocytic cells. J Med Microbiol 2005; 54:225–233.
77. Jang AR, Kang MJ, Shin JI, Kwon SW, Park JY, Ahn JH, Lee TS, Kim DY, Choi BG, Seo MW, Yang SJ, Shin MK, Park JH. Unveiling the crucial role of type IV secretion system and motility of *Helicobacter pylori* in IL-1beta production via NLRP3 inflammasome activation in neutrophils. Front Immunol 2020;11:1121.
78. Satin B, Del Giudice G, Della Bianca V, Dusi S, Laudanna C, Tonello F, Kelleher D, Rappuoli R, Montecucco C, Rossi F. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. J Exp Med 2000; 191:1467–1476.
79. Otani K, Tanigawa T, Watanabe T, Nadatani Y, Sogawa M, Yamagami H, Shiba M, Watanabe K, Tominaga K, Fujiwara Y, Arakawa T. Toll-like receptor 9 signaling has anti-inflammatory effects on the early phase of *Helicobacter pylori*-induced gastritis. Biochem Biophys Res Commun 2012;426:342–349.
80. Mai UE, Perez-Perez GI, Allen JB, Wahl SM, Blaser MJ, Smith PD. Surface proteins from *Helicobacter pylori* exhibit chemotactic activity for human leukocytes and are present in gastric mucosa. J Exp Med 1992; 175:517–525.
81. Ito T, Kobayashi D, Uchida K, Takemura T, Nagaoka S, Kobayashi I, Yokoyama T, Ishige I, Ishige Y, Ishida N, Furukawa A, Muraoka H, Ikeda S, Sekine M, Ando N,

Suzuki Y, Yamada T, Suzuki T, Eishi Y. *Helicobacter pylori* invades the gastric mucosa and translocates to the gastric lymph nodes. Lab Invest 2008;88:664–681.

82. 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 M, Appelmelk BJ, Yamasaki S. *Helicobacter pylori* metabolites exacerbate gastritis through C-type lectin receptors. J Exp Med 2021;218:e20200815.
83. Guiney DG, Hasegawa P, Cole SP. *Helicobacter pylori* preferentially induces interleukin 12 (IL-12) rather than IL-6 or IL-10 in human dendritic cells. Infect Immun 2003; 71:4163–4166.
84. 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.
85. Odenbreit S, Gebert B, Puls J, Fischer W, Haas R. Interaction of *Helicobacter pylori* with professional phagocytes: role of the *cag* pathogenicity island and translocation, phosphorylation and processing of CagA. Cell Microbiol 2001;3:21–31.
86. Gobert AP, Verriere T, Asim M, Barry DP, Piazuelo MB, de Sablet T, Delgado AG, Bravo LE, Correa P, Peek RM Jr, Chaturvedi R, Wilson KT. Heme oxygenase-1 dysregulates macrophage polarization and the immune response to *Helicobacter pylori*. J Immunol 2014; 193:3013–3022.
87. Tanaka H, Yoshida M, Nishiumi S, Ohnishi N, Kobayashi K, Yamamoto K, Fujita T, Hatakeyama M, Azuma T. The CagA protein of *Helicobacter pylori* suppresses the functions of dendritic cell in mice. Arch Biochem Biophys 2010;498:35–42.
88. Ko SH, Rho DJ, Jeon JI, Kim YJ, Woo HA, Kim N, Kim JM. Crude preparations of *Helicobacter pylori* outer membrane vesicles induce upregulation of heme oxygenase-1 via activating Akt-Nrf2 and mTOR-IkappaB kinase-NF-kappaB pathways in dendritic cells. Infect Immun 2016;84:2162–2174.
89. He H, Liu J, Li L, Qian G, Hao D, Li M, Zhang Y, Hong X, Xu J, Yan D. *Helicobacter pylori* CagA Interacts with shp-1 to suppress the immune response by targeting TRAF6 for K63-linked ubiquitination. J Immunol 2021; 206:1161–1170.
90. Kaebisch R, Mejias-Luque R, Prinz C, Gerhard M. *Helicobacter pylori* cytotoxin-associated gene A impairs human dendritic cell maturation and function through IL-10-mediated activation of STAT3. J Immunol 2014; 192:316–323.
91. Sarajlic M, Neuper T, Vetter J, Schaller S, Klicznik MM, Gratz IK, Wessler S, Posselt G, Horejs-Hoeck JH. *pylori* modulates DC functions via T4SS/TNFalpha/p38-dependent SOCS3 expression. Cell Commun Signal 2020;18:160.
92. Lofgren JL, Whary MT, Ge Z, Muthupalani S, Taylor NS, Mobley M, Potter A, Varro A, Eibach D, Suerbaum S, Wang TC, Fox JG. Lack of commensal flora in *Helicobacter pylori*-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. Gastroenterology 2011;140:210–220.
93. Rolig AS, Cech C, Ahler E, Carter JE, Ottemann KM. The degree of *Helicobacter pylori*-triggered inflammation is manipulated by preinfection host microbiota. Infect Immun 2013;81:1382–1389.
94. Jones TA, Hernandez DZ, Wong ZC, Wandler AM, Guillemin K. The bacterial virulence factor CagA induces microbial dysbiosis that contributes to excessive epithelial cell proliferation in the *Drosophila* gut. PLoS Pathog 2017;13:e1006631.
95. Tan MP, Kaparakis M, Galic M, Pedersen J, Pearse M, Wijburg OL, Janssen PH, Strugnell RA. Chronic *Helicobacter pylori* infection does not significantly alter the microbiota of the murine stomach. Appl Environ Microbiol 2007;73:1010–1013.
96. Pan M, Wan C, Xie Q, Huang R, Tao X, Shah NP, Wei H. Changes in gastric microbiota induced by *Helicobacter pylori* infection and preventive effects of *Lactobacillus plantarum* ZDY 2013 against such infection. J Dairy Sci 2016;99:970–981.
97. Kienesberger S, Cox LM, Livanos A, Zhang XS, Chung J, Perez-Perez GI, Gorkiewicz G, Zechner EL, Blaser MJ. Gastric *Helicobacter pylori* infection affects local and distant microbial populations and host responses. Cell Rep 2016;14:1395–1407.
98. Ge Z, Sheh A, Feng Y, Muthupalani S, Ge L, Wang C, Kurnick S, Mannion A, Whary MT, Fox JG. *Helicobacter pylori*-infected C57BL/6 mice with different gastrointestinal microbiota have contrasting gastric pathology, microbial and host immune responses. Sci Rep 2018; 8:8014.
99. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, Perez-Perez G, Blaser MJ, Relman DA. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A 2006; 103:732–737.
100. Li XX, Wong GL, To KF, Wong VW, Lai LH, Chow DK, Lau JY, Sung JJ, Ding C. Bacterial microbiota profiling in gastritis without *Helicobacter pylori* infection or non-steroidal anti-inflammatory drug use. PLoS One 2009; 4:e7985.
101. Engstrand L, Lindberg M. *Helicobacter pylori* and the gastric microbiota. Best Pract Res Clin Gastroenterol 2013;27:39–45.
102. Yang I, Woltemate S, Piazuelo MB, Bravo LE, Yepez MC, Romero-Gallo J, Delgado AG, Wilson KT, Peek RM, Correa P, Josenhans C, Fox JG, Suerbaum S. Different gastric microbiota compositions in two human populations with high and low gastric cancer risk in Colombia. Sci Rep 2016;6:18594.
103. Maldonado-Contreras A, Goldfarb KC, Godoy-Vitorino F, Karaoz U, Contreras M, Blaser MJ, Brodie EL, Dominguez-Bello MG. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. ISME J 2011;5:574–579.
104. Brawner KM, Kumar R, Serrano CA, Ptacek T, Lefkowitz E, Morrow CD, Zhi D, Kyanam-Kabir-Baig KR, Smythies LE, Harris PR, Smith PD. *Helicobacter pylori*

infection is associated with an altered gastric microbiota in children. Mucosal Immunol 2017;10:1169–1177.
105. Zheng W, Miao J, Luo L, Long G, Chen B, Shu X, Gu W, Peng K, Li F, Zhao H, Botchway BOA, Fang M, Jiang M. The effects of *Helicobacter pylori* infection on microbiota associated with gastric mucosa and immune factors in children. Front Immunol 2021;12:625586.
106. Kim EJ, Lee JR, Chung WC, Jung SH, Sung HJ, Lee YW, Oh YS, Kim SB, Paik CN, Lee KM, Noh SJ. Association between genetic polymorphisms of NOD 1 and *Helicobacter pylori*-induced gastric mucosal inflammation in healthy Korean population. Helicobacter 2013;18:143–150.
107. Gonzalez-Hormazabal P, Pelaez D, Musleh M, Bustamante M, Stambuk J, Pisano R, Valladares H, Lanzarini E, Chiong H, Suazo J, Quinones LA, Varela NM, Castro VG, Jara L, Berger Z. NOD1 rs2075820 (p.E266K) polymorphism is associated with gastric cancer among individuals infected with *cag*PAI-positive *H. pylori*. Biol Res 2021;54:13.
108. Hofner P, Gyulai Z, Kiss ZF, Tiszai A, Tiszlavicz L, Toth G, Szoke D, Molnar B, Lonovics J, Tulassay Z, Mandi Y. Genetic polymorphisms of NOD1 and IL-8, but not polymorphisms of TLR4 genes, are associated with *Helicobacter pylori*-induced duodenal ulcer and gastritis. Helicobacter 2007;12:124–131.
109. Rosenstiel P, Hellmig S, Hampe J, Ott S, Till A, Fischbach W, Sahly H, Lucius R, Folsch UR, Philpott D, Schreiber S. Influence of polymorphisms in the NOD1/CARD4 and NOD2/CARD15 genes on the clinical outcome of *Helicobacter pylori* infection. Cell Microbiol 2006;8:1188–1198.
110. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599–603.
111. Hold GL, Rabkin CS, Chow WH, Smith MG, Gammon MD, Risch HA, Vaughan TL, McColl KE, Lissowska J, Zatonski W, Schoenberg JB, Blot WJ, Mowat NA, Fraumeni JF Jr, El-Omar EM. A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. Gastroenterology 2007;132:905–912.
112. Kupcinskas J, Wex T, Bornschein J, Selgrad M, Leja M, Juozaityte E, Kiudelis G, Jonaitis L, Malfertheiner P. Lack of association between gene polymorphisms of Angiotensin converting enzyme, Nod-like receptor 1, Toll-like receptor 4, FAS/FASL and the presence of *Helicobacter pylori*-induced premalignant gastric lesions and gastric cancer in Caucasians. BMC Med Genet 2011;12:112.
113. Trejo-de la OA, Torres J, Perez-Rodriguez M, Camorlinga-Ponce M, Luna LF, Abdo-Francis JM, Lazcano E, Maldonado-Bernal C. *TLR4* single-nucleotide polymorphisms alter mucosal cytokine and chemokine patterns in Mexican patients with *Helicobacter pylori*-associated gastroduodenal diseases. Clin Immunol 2008;129:333–340.
114. Kato I, Canzian F, Plummer M, Franceschi S, van Doorn LJ, Vivas J, Lopez G, Lu Y, Gioia-Patricola L, Severson RK, Schwartz AG, Munoz N. Polymorphisms in genes related to bacterial lipopolysaccharide/peptidoglycan signaling and gastric precancerous lesions in a population at high risk for gastric cancer. Dig Dis Sci 2007;52:254–261.
115. Garza-Gonzalez E, Bosques-Padilla FJ, Mendoza-Ibarra SI, Flores-Gutierrez JP, Maldonado-Garza HJ, Perez-Perez GI. Assessment of the toll-like receptor 4 Asp299Gly, Thr399Ile and interleukin-8 -251 polymorphisms in the risk for the development of distal gastric cancer. BMC Cancer 2007;7:70.
116. Achyut BR, Ghoshal UC, Moorchung N, Mittal B. Association of Toll-like receptor-4 (Asp299Gly and Thr399Ileu) gene polymorphisms with gastritis and precancerous lesions. Hum Immunol 2007;68:901–907.
117. Bagheri N, Azadegan-Dehkordi F, Sanei H, Taghikhani A, Rahimian G, Salimzadeh L, Hashemzadeh-Chaleshtori M, Rafieian-Kopaei M, Shirzad M, Shirzad H. Associations of a *TLR4* single-nucleotide polymorphism with *H. pylori* associated gastric diseases in Iranian patients. Clin Res Hepatol Gastroenterol 2014;38:366–371.
118. Bagheri N, Azadegan-Dehkordi F, Rahimian G, Hashemzadeh-Chaleshtori M, Rafieian-Kopaei M, Kheiri S, Gholipour A, Shirzad H. Altered Th17 cytokine expression in *Helicobacter pylori* patients with *TLR4* (D299G) polymorphism. Immunol Invest 2016;45:161–171.
119. Fan YF, Wu YM, Liu H, Yu Y, Jiang YY, Xue YZ, Liu ZL, Wei MX. TLR4 polymorphisms associated with developing gastric pre-cancer lesions in a Chinese Han population. Hum Immunol 2014;75:176–181.
120. Hishida A, Matsuo K, Goto Y, Mitsuda Y, Hiraki A, Naito M, Wakai K, Tajima K, Hamajima N. Toll-like receptor 4 +3725 G/C polymorphism, *Helicobacter pylori* seropositivity, and the risk of gastric atrophy and gastric cancer in Japanese. Helicobacter 2009;14:47–53.
121. Higgins SC, Lavelle EC, McCann C, Keogh B, McNeela E, Byrne P, O'Gorman B, Jarnicki A, McGuirk P, Mills KH. Toll-like receptor 4-mediated innate IL-10 activates antigen-specific regulatory T cells and confers resistance to *Bordetella pertussis* by inhibiting inflammatory pathology. J Immunol 2003;171:3119–3127.
122. Brand S, Staudinger T, Schnitzler F, Pfennig S, Hofbauer K, Dambacher J, Seiderer J, Tillack C, Konrad A, Crispin A, Goke B, Lohse P, Ochsenkuhn T. The role of Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms and *CARD15/NOD2* mutations in the susceptibility and phenotype of Crohn's disease. Inflamm Bowel Dis 2005;11:645–652.
123. Ng MT, Van't Hof R, Crockett JC, Hope ME, Berry S, Thomson J, McLean MH, McColl KE, El-Omar EM, Hold GL. Increase in NF-kappaB binding affinity of the variant C allele of the toll-like receptor 9 -1237T/C polymorphism is associated with *Helicobacter pylori*-induced gastric disease. Infect Immun 2010;78:1345–1352.
124. Hold GL, Rabkin CS, Gammon MD, Berry SH, Smith MG, Lissowska J, Risch HA, Chow WH, Mowat NA,

Vaughan TL, El-Omar EM. *CD14*-159C/T and *TLR9*-1237T/C polymorphisms are not associated with gastric cancer risk in Caucasian populations. Eur J Cancer Prev 2009;18:117–119.

125. Susi MD, Lourenco Caroline M, Rasmussen LT, Payao SLM, Rossi AFT, Silva AE, de Oliveira-Cucolo JG. Toll-like receptor 9 polymorphisms and *Helicobacter pylori* influence gene expression and risk of gastric carcinogenesis in the Brazilian population. World J Gastrointest Oncol 2019;11:998–1010.
126. Loganathan R, Nazeer M, Goda V, Devaraju P, Ali M, Karunakaran P, Jayaraman M. Genetic variants of *TLR4* and *TLR9* are risk factors for chronic *Helicobacter pylori* infection in South Indian Tamils. Hum Immunol 2017; 78:216–220.
127. Trejo-de la OA, Torres J, Sanchez-Zauco N, Perez-Rodriguez M, Camorlinga-Ponce M, Flores-Luna L, Lazcano-Ponce E, Maldonado-Bernal C. Polymorphisms in *TLR9* but not in *TLR5* increase the risk for duodenal ulcer and alter cytokine expression in the gastric mucosa. Innate Immun 2015;21:706–713.
128. Ohyauchi M, Imatani A, Yonechi M, Asano N, Miura A, Iijima K, Koike T, Sekine H, Ohara S, Shimosegawa T. The polymorphism interleukin 8 -251 A/T influences the susceptibility of *Helicobacter pylori* related gastric diseases in the Japanese population. Gut 2005;54:330–335.
129. Taguchi A, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, Niwa Y, Goto H. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. Cancer Epidemiol Biomarkers Prev 2005;14:2487–2493.
130. Ye BD, Kim SG, Park JH, Kim JS, Jung HC, Song IS. The interleukin-8-251 A allele is associated with increased risk of noncardia gastric adenocarcinoma in *Helicobacter pylori*-infected Koreans. J Clin Gastroenterol 2009;43:233–239.
131. Song JH, Kim SG, Jung SA, Lee MK, Jung HC, Song IS. The interleukin-8-251 AA genotype is associated with angiogenesis in gastric carcinogenesis in *Helicobacter pylori*-infected Koreans. Cytokine 2010;51:158–165.
132. Zhang L, Du C, Guo X, Yuan L, Niu W, Yu W, Er L, Wang S. Interleukin-8-251A/T polymorphism and *Helicobacter pylori* infection influence risk for the development of gastric cardiac adenocarcinoma in a high-incidence area of China. Mol Biol Rep 2010; 37:3983–3989.
133. Szoke D, Molnar B, Solymosi N, Klausz G, Gyulai Z, Toth B, Mandi Y, Tulassay Z. T-251A polymorphism of *IL-8* relating to the development of histological gastritis and G-308A polymorphism of *TNF-alpha* relating to the development of macroscopic erosion. Eur J Gastroenterol Hepatol 2008;20:191–195.
134. Lee WP, Tai DI, Lan KH, Li AF, Hsu HC, Lin EJ, Lin YP, Sheu ML, Li CP, Chang FY, Chao Y, Yen SH, Lee SD. The -251T allele of the interleukin-8 promoter is associated with increased risk of gastric carcinoma featuring diffuse-type histopathology in Chinese population. Clin Cancer Res 2005;11:6431–6441.
135. de Oliveira JG, Rossi AF, Nizato DM, Cadamuro AC, Jorge YC, Valsechi MC, Venancio LP, Rahal P, Pavarino EC, Goloni-Bertollo EM, Silva AE. Influence of functional polymorphisms in *TNF-alpha, IL-8*, and *IL-10* cytokine genes on mRNA expression levels and risk of gastric cancer. Tumour Biol 2015;36:9159–9170.
136. Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M, Prinz C. Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. Gut 2004; 53:1082–1089.
137. Tseng FC, Brown EE, Maiese EM, Yeager M, Welch R, Gold BD, Owens M, Cranston B, Hanchard B, El-Omar E, Hisada M. Polymorphisms in cytokine genes and risk of *Helicobacter pylori* infection among Jamaican children. Helicobacter 2006;11:425–430.
138. Hou L, El-Omar EM, Chen J, Grillo P, Rabkin CS, Baccarelli A, Yeager M, Chanock SJ, Zatonski W, Sobin LH, Lissowska J, Fraumeni JF Jr, Chow WH. Polymorphisms in Th1-type cell-mediated response genes and risk of gastric cancer. Carcinogenesis 2007; 28:118–123.
139. Hnatyszyn A, Wielgus K, Kaczmarek-Rys M, Skrzypczak-Zielinska M, Szalata M, Mikolajczyk-Stecyna J, Stanczyk J, Dziuba I, Mikstacki A, Slomski R. Interleukin-1 gene polymorphisms in chronic gastritis patients infected with *Helicobacter pylori* as risk factors of gastric cancer development. Arch Immunol Ther Exp (Warsz) 2013;61:503–512.
140. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000; 404:398–402.
141. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF Jr, Chow WH. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology 2003;124:1193–1201.
142. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, Amorim A, Seruca R, Caldas C, Carneiro F, Sobrinho-Simoes M. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology 2001;121:823–829.
143. Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinha AF, Quint W, Caldas C, van Doorn LJ, Carneiro F, Sobrinho-Simoes M. *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. J Natl Cancer Inst 2002;94:1680–1687.
144. Zeng ZR, Hu PJ, Hu S, Pang RP, Chen MH, Ng M, Sung JJ. Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. Gut 2003;52:1684–1689.
145. Zambon CF, Basso D, Navaglia F, Germano G, Gallo N, Milazzo M, Greco E, Fogar P, Mazza S, Di Mario F, Basso G, Rugge M, Plebani M. *Helicobacter pylori* virulence genes and host IL-1RN and IL-1beta genes

interplay in favouring the development of peptic ulcer and intestinal metaplasia. Cytokine 2002;18:242–251.
146. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, Yamaoka Y. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in *Helicobacter pylori* infection. Gastroenterology 2002;123:1793–1803.
147. Yea SS, Yang YI, Jang WH, Lee YJ, Bae HS, Paik KH. Association between TNF-alpha promoter polymorphism and *Helicobacter pylori cagA* subtype infection. J Clin Pathol 2001;54:703–706.
148. Jang WH, Yang YI, Yea SS, Lee YJ, Chun JH, Kim HI, Kim MS, Paik KH. The -238 tumor necrosis factor-alpha promoter polymorphism is associated with decreased susceptibility to cancers. Cancer Lett 2001; 166:41–46.
149. Machado JC, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simoes M. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. Gastroenterology 2003;125:364–371.
150. Lu W, Pan K, Zhang L, Lin D, Miao X, You W. Genetic polymorphisms of interleukin (IL)-1B, IL-1RN, IL-8, IL-10 and tumor necrosis factor {alpha} and risk of gastric cancer in a Chinese population. Carcinogenesis 2005; 26:631–636.
151. Hellmig S, Hampe J, Folsch UR, Schreiber S. Role of IL-10 promoter haplotypes in *Helicobacter pylori* associated gastric inflammation. Gut 2005;54:888.
152. Wu MS, Wu CY, Chen CJ, Lin MT, Shun CT, Lin JT. Interleukin-10 genotypes associate with the risk of gastric carcinoma in Taiwanese Chinese. Int J Cancer 2003;104:617–623.
153. Sugimoto M, Furuta T, Shirai N, Nakamura A, Kajimura M, Sugimura H, Hishida A. Effects of interleukin-10 gene polymorphism on the development of gastric cancer and peptic ulcer in Japanese subjects. J Gastroenterol Hepatol 2007;22:1443–1449.
154. Sicinschi LA, Lopez-Carrillo L, Camargo MC, Correa P, Sierra RA, Henry RR, Chen J, Zabaleta J, Piazuelo MB, Schneider BG. Gastric cancer risk in a Mexican population: role of *Helicobacter pylori* CagA positive infection and polymorphisms in interleukin-1 and -10 genes. Int J Cancer 2006;118:649–657.
155. Kato I, Canzian F, Franceschi S, Plummer M, van Doorn LJ, Lu Y, Gioia-Patricola L, Vivas J, Lopez G, Severson RK, Schwartz AG, Munoz N. Genetic polymorphisms in anti-inflammatory cytokine signaling and the prevalence of gastric precancerous lesions in Venezuela. Cancer Causes Control 2006;17:1183–1191.
156. Won HH, Kim JW, Kim MJ, Kim S, Park JH, Lee KA. Interleukin 10 polymorphisms differentially influence the risk of gastric cancer in East Asians and Caucasians. Cytokine 2010;51:73–77.
157. Kang JM, Kim N, Lee DH, Park JH, Lee MK, Kim JS, Jung HC, Song IS. The effects of genetic polymorphisms of IL-6, IL-8, and IL-10 on *Helicobacter pylori*-induced gastroduodenal diseases in Korea. J Clin Gastroenterol 2009;43:420–428.
158. Murphy G, Thornton J, McManus R, Swan N, Ryan B, Hughes DJ, O'Morain CA, O'Sullivan M. Association of gastric disease with polymorphisms in the inflammatory-related genes *IL-1B, IL-1RN, IL-10, TNF* and *TLR4*. Eur J Gastroenterol Hepatol 2009;21:630–635.

Received October 21, 2021. Accepted January 25, 2022.

Correspondence
Address correspondence to: Alain P. Gobert, Vanderbilt University Medical Center, 2215B Garland Ave, 1030C MRB IV, Nashville, TN 37232-0252. e-mail: alain.p.gobert@vumc.org; tel: (615) 343-5577. or Keith T. Wilson, Vanderbilt University Medical Center, 2215B Garland Ave, 1015C MRB IV, Nashville, TN 37232-0252. e-mail: keith.wilson@vumc.org; tel: (615) 343-5675.

Conflicts of interest
The authors disclose no conflicts.

Funding
This work was funded by National Institutes of Health grants R01DK128200, R01CA190612, P01CA116087, P01CA028842, and R41CA257262 (KTW); Veterans Affairs Merit Review grants I01BX001453 and I01CX002171 (KTW); Senior Research Award 703003 from the Crohn's & Colitis Foundation (APG and KTW); Department of Defense Grant W81XWH-21-1-0617 (KTW), the Thomas F. Frist Sr. Endowment (KTW); and the Vanderbilt Center for Mucosal Inflammation and Cancer (KTW).