REVIEW

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

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SUMMARY

Helicobacter pylori is a pathogen that causes gastric cancer by stimulating inflammation. The innate 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)

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Helicobacter pylori is a Gram-negative bacterium that colonizes the stomach of 4.4 billion people.\textsuperscript{1} Its persistence in the stomach causes chronic gastritis, which can progress to intestinal metaplasia (IM), and gastric adenocarcinoma,\textsuperscript{2} the fourth leading cause of cancer mortality.\textsuperscript{3} Over 100,000 years of coevolution between Helicobacter pylori and humans\textsuperscript{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 (cagPAI), which carries genes encoding for a functional type 4 secretion system (T4SS) and the virulence factor CagA.\textsuperscript{5–7} CagA is injected into the cytoplasm of GECs by the T4SS,\textsuperscript{7} phosphorylated on tyrosine residues of Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs by SRC and then ABL kinases,\textsuperscript{8,9} and binds/activates SHP2,\textsuperscript{10} which signals through ERK1/2 to induce cytoskeletal rearrangements and increased motility linked to carcinogenesis.\textsuperscript{12,13} Native CagA can also modulate cellular functions.\textsuperscript{14} Large epidemiological studies have correlated cagA$^+$ strains to higher rates of gastric cancer (GC).\textsuperscript{15,16}

Numerous studies have shown that multiple GEC lines produce more interleukin (IL)-8 when stimulated with H. pylori strains harboring the cagPAI compared with cagPAI$^-$ isolates.\textsuperscript{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-$\alpha$), for example, are less expressed in KATO-3 cells infected with cagPAI$^+$ compared with cagPAI$^-$ isolates.\textsuperscript{21} Thus, effectors encoded by the cagPAI have been implicated in the induction of innate responses by GECs.
H. pylori mutants for cagE, encoding for an ATPase of the T4SS, lose their ability to stimulate ERK1/2, p38 and JNK phosphorylation, nuclear factor kappa B (NF-κB) NF-κB activation, and to induce IL-8 or other chemokines, such as CCL2 or CCL5. 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. Deletion of one of the cagPAI genes involved in T4SS structure or function is sufficient to reduce the production of IL-8. Also, cagPAI− strains induce greater phosphorylation of epidermal growth factor receptor than cagPAI+ strains in AGS cells, and a cagE mutant did not activate epidermal growth factor receptor. 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. 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. Similar, 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. 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−. Interestingly, Brandt et al identified 2 distinct groups of H. pylori carrying a functional cagPAI: the high and the low IL-8 inducers. 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. 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; this was associated with CagA-mediated stimulation of the RAS/ERK1/2/NF-κB pathway.

Further, it has been reported that CagA phosphorylation is required to activate innate responses of GECs. Although the number of EPIYA motifs, and thus CagA phosphorylation level, has been reported to have no effect on IL-8 secretion, 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. 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; 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. 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. NF-κB induction and IL-8 production are reduced when AGS cells are stimulated with a mutant deficient in lytic trans-glycosylase activity (Dslt) that releases less peptidoglycan (PG) muropeptides than WT H. pylori. 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. This mechanism was also implicated in stimulation of MAP kinases p38 and ERK1/2, and the transcription factor AP-1, and induction of beta-defensin 2 expression by H. pylori-infected AGS cells. Interestingly, induction of IL-3 by H. pylori in AGS cells depends on both NOD1 signaling and CagA translocation, demonstrating that H. pylori can exploit its T4SS to stimulate various pathways simultaneously. Note that H. pylori outer membrane vesicles, irrespective of their cagPAI status, can also deliver PG in GECs and induce NOD1 signaling and NOD1/RIP2-mediated autophagy.

Increased colonization by cagPAI+ strains was reported in Nod1−/− mice compared with WT animals at 7 and 30 days post-inoculation. Of importance, the colonization by H. felis, a bacterium lacking a cagPAI 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. 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. These authors also observed spontaneous dysplasia in INS-GAS mice with Nod1 deletion, which was further increased by H. pylori infection. In contrast, infection of Mongolian gerbils with H. pylori DpgdA, 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. 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, evidencing that the T4SS can also be used by H. pylori to...
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. A cagb mutant that cannot translocate CagA is still able to stimulate TLR9, 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, injection of DNA can be considered as an adaptation of *H. pylori* to the response of the mucosa.

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. αCAG, cholesteryl acyl α-glucoside; αCPG, cholesteryl phosphatidyl α-glucoside; CLC4E, macrophage-inducible C-type lectin receptor.
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 *cagPAI* does not affect IL-8 production. 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. 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, 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.

**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, 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. In contrast, the UreB subunit directly binds to CD74 and stimulates NF-κB activation and IL-8 synthesis by N87 cells (Figure 1). Notably, in these last two 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 or by autolysis, 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 flagellum. Highly motile clinical strains induce more IL-8 production than bacteria with low motility. 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. 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. 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. 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; however, production of IL-8, GRO-α, and MIP-3α was not observed, 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 or human primary GECs 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.

**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. 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. Functional OipA is associated with high levels of *H. pylori* colonization, neutrophil infiltration, and mucosal IL-8 in humans and mice. Interestingly, CagA translocation and cytokine induction in AGS cells are not affected by *oipA* deletion after a 4-hour infection or with a high multiplicity of infection (1000), 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 *cagPAI* have a complementary effect on activation of GECs: (1) phosphorylation of AKT1 at Ser473 and Thr308 is dependent on OipA and *cagPAI*, respectively, and the PI3K-AKT pathway is required for full activation of IL-8; (2) whereas the *cagPAI* 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; (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 *cagPAI*-dependent activation of NF-κB; and (4) activation of p38 by OipA, and AP-1 and NF-κB by the *cagPAI* is required for IL-6 production by GECs.

Lastly, other outer membrane proteins such as AlpAB or HomB 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 Hsp6075,76 flagellin,77 NapA,79 or DNA79 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.77,78,84 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. 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 macrophages86 and DCs.89 In macrophages, heme oxygenase-1 dampens the proinflammatory/antibacterial M1 response and increases the anti-inflammatory Mreg phenotype.90 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.91 The authors found that CagA in macrophages interacts with SHP1, thus inhibiting polyubiquitination and signaling of TRAF6.92 Similarly, DCs infected with H. pylori DcagA produce more TNF-α and IL-12p40, but less IL-10, than the WT strain.97,90 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 triad 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, and A. pasteurianus and Lactobacillus brevis are both dominant in CagA-transgenic flies. Notably, (1) the microbe 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, 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 monospecies association 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; (2) a prevalence of Proteobacteria, including the families Pasteurellaceae, Erysipelotrichaceae, and...
**Halomonadaceae**, in specific-pathogen-free C57BL/6J mice; and (3) a reduced abundance of Bacteroidetes and Firmicutes in C57BL/6 animals. 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 Bacteroidetes and a reduction of Actinobacteria and antibiotics results in an increase of cluster IV and XIVa Firmicutes in C57BL/6 animals. 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 Bacteroidetes and a reduction of Actinobacteria and antibiotics results in an increase of cluster IV and XIVa Firmicutes in C57BL/6 animals.

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). In addition, the phyla Deferribacteres and Verrucomicrobia are only present in Tac mice. Moreover, the microbial community of the stomach of Jax mice, but not Tac mice, was significantly altered by *H. pylori* infection. Concomitantly, the *H. pylori*-induced expression of Il1b, Il17a, and Reg3g was higher in Jax vs Tac mice. 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, Neisseria, Haemophilus, and Rothia. Overall, there is no difference between the antrum and corpus region. 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, others have reported a reduction of the relative abundance of Proteobacteria and an increase of the Streptococcus genus in infected Chinese patients as well as higher abundance of Proteobacteria and Acidobacteria in Amerindian subjects. 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. 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. 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. 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. 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 cagPAI. 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 cagPAI+ *H. pylori*. Duodenal ulceration is also significantly associated with this homozygous variation in Hungarian individuals.

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

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        |
|        | +1196C>T     | Mexican           | ↑ DU                     | 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        |
|        | −845T>C      | East European     | ↑ DU                     | 110        |
|        |              | Mexican           | ↑ GC                     | 117        |
|        |              | Chinese           | ↑ GC                     | 136        |
|        |              | Brazilian         | ↑ Gastritis, ↑ GC        | 137        |
| IL1A   | −899C>T      | Jamaican          | ↓ Infection              | 139        |
| IL1B   | −31C>T       | Caucasian         | ↑ GC                     | 142, 143   |
|        | −511C>T      | Italian           | ↑ IM                     | 147        |
| IL1RN  | IL1RN*2      | 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        |
| 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. However, GC is not related to the TLR4+3725G>C polymorphism in individuals from Germany, Lithuania, and Latvia.

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. 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. But overall, the TLR4+896A>Г and/or TLR4+1196C>T variants are more frequent among infected patients with duodenal ulcer and GC than in patients with gastritis.

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.

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. However, this last variant is not associated with risk for GC.

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. 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.

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

Overall, the loss of TLR4 function 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. Notably, TLR4 polymorphisms are associated with exacerbated inflammation in patients with Crohn’s disease.

**TLR9**

The TLR9–1237T>C promoter polymorphisms creates a potential NF-κB binding site and thus increases the transcription of TLR9. 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, though not with GC. In contrast, this polymorphism is associated with a higher risk of gastric carcinogenesis in patients from Brazil. The A allele at position 2848 is associated with long-term infection by H. pylori in Indian Tamils and is more frequent in Mexican patients with duodenal ulcer than in the group with non-atrophic gastritis.

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. 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. 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, is associated with higher gastric neutrophil infiltration, atrophy, IM, and GC compared with the A/T or T/T genotypes. The same A/A genotype in European Caucasian patients is significantly increased in patients with gastritis, but is not correlated with GC. 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. The A/T genotype correlates with duodenal ulcer in H. pylori-infected subjects from Eastern Europe and with GC in patients from Mexico. Intriguingly, the CXCL8–251T>C 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.

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. 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.

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/IL1B–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 vacAs1+, vacAm1+, 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 IL10−1082G/G or IL10−592A/C/C genotypes, respectively.156 Further, IL10 mRNA expression is lower in carriers of the IL10−1082A/−819T/−592A haplotype compared with the GCC carriers.153 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.153 A second study performed in Germany showed no association between IL10 polymorphisms and gastric ulceration.154

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 Taiwanese152 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,
the combination of the carriage of \( IL1B \sim 511T \), \( IL1RN^*2 \) homozygosity (high IL-1\( \beta \)), and TNF\( \sim 308G \) (high TNF-\( \alpha \)) 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 \( IL10 \sim 592A/A \) genotype (low IL-10) and the \( CXCL8 \sim 251A/A \) 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.

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