How host regulation of *Helicobacter pylori*-induced gastritis protects against peptic ulcer disease and gastric cancer

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Dhar P, Ng GZ, Sutton P. How host regulation of *Helicobacter pylori*-induced gastritis protects against peptic ulcer disease and gastric cancer. *Am J Physiol Gastrointest Liver Physiol* 311: G514–G520, 2016. First published July 28, 2016; doi:10.1152/ajpgi.00146.2016.—The bacterial pathogen *Helicobacter pylori* is the etiological agent of a range of gastrointestinal pathologies including peptic ulcer disease and the major killer, gastric adenocarcinoma. Infection with this bacterium induces a chronic inflammatory response in the gastric mucosa (gastritis). It is this gastritis that, over decades, eventually drives the development of *H. pylori*-associated disease in some individuals. The majority of studies investigating *H. pylori* pathogenesis have focused on factors that promote disease development in infected individuals. However, an estimated 85% of those infected with *H. pylori* remain completely asymptomatic, despite the presence of pathogenic bacteria that drive a chronic gastritis that lasts many decades. This indicates the presence of highly effective regulatory processes in the host that, in most cases, keeps a check on inflammation and protect against disease. In this minireview we discuss such known host factors and how they prevent the development of *H. pylori*-associated pathologies.

Helicobacter pylori; host factors; gastritis; immune suppression

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HELICOBACTER PYLORI ARE PATHOGENIC, highly motile, microaerophilic bacteria that colonize the mucus layer lining the human stomach. This highly prevalent infection is usually acquired in childhood and, without antibiotic treatment, will persist throughout the lifetime of the individual, inducing an inflammatory immune response in the gastric mucosa (gastritis). It is this gastritis that, over decades of chronic bacterial-induced immunostimulation, eventually drives the development of *H. pylori*-associated disease in some individuals. *H. pylori* infection is the proven cause of a range of gastrointestinal pathologies, including peptic ulcer disease (gastric and duodenal ulcers) (35), gastric mucosa-associated lymphoid tissue (MALT) lymphoma (63), and gastric adenocarcinoma (60). Due to its high prevalence, particularly in Asia, gastric adenocarcinoma is globally the 3rd leading cause of death due to cancer (22).

The oft-quoted statistic is that ~15% of those infected with *H. pylori* develop an associated pathology; the individuals who do develop such a disease are believed those who mount the most severe inflammatory response to this infection. The reason why some individuals develop a more severe gastritis and progress to disease are multifold and include infection by more virulent strains of *H. pylori*, the impact of environmental factors such as high-salt diet and smoking, as well as the effect of host genetic factors that make certain unfortunate individu-

als more susceptible to the pathological sequelae of this infection (reviewed in Ref. 64). Understandably, the majority of studies investigating *H. pylori* pathogenesis have focused on factors that promote disease in infected individuals, and these have been the subject of numerous previous reviews.

Rarely considered, however, is the other side of the coin. It is a quite remarkable feat perhaps, that the vast majority (an estimated 85%) of people infected with *H. pylori* remain completely asymptomatic for life, despite the presence of pathogenic bacteria inducing a chronic inflammation over a period of many decades (often 70–80 years). This predominant lack of pathology clearly indicates the presence of highly effective regulatory processes that, at least in most individuals, protect against the pathological consequence of *H. pylori* infection. It is not that asymptomatic individuals do not develop gastritis — to our knowledge no *H. pylori*-infected person has ever been reported who does not have gastritis (13) — it is just that the severity of the inflammation can be regulated to a degree that prevents the development of pathology.

For *H. pylori* to induce gastritis, they must first swim through the mucus layers lining the stomach to reach the surface of the gastric epithelial cells, where a proportion of them attach. From this locale, these bacteria trigger the host innate immune system, with a range of consequences including the opening of the tight cell-cell junctions that normally maintain the impermeable epithelial barrier and the recruitment of proinflammatory cells including neutrophils and macrophages to the gastric mucosa (1). This is subsequently followed by

activation of the adaptive immune response where both Th1- and Th17-type T helper cells have been associated with the development of chronic gastritis (2, 54).

It is worth mentioning here that, to coevolve with the host, *H. pylori* has successfully managed to dampen the immune response elicited against it. Compared with other gram-negative bacteria, *H. pylori* lipopolysaccharide and flagella are poor activators of the innate signaling pathway mediated via recognition of these bacterial ligands by their specific Toll-like receptors (TLR), making them less immunogenic (51). Additionally, two *H. pylori* virulence factors, vacuolating cytotoxin A and γ-glutamyl transpeptidase, promote the development of regulatory T cells (discussed later) to limit inflammation (51), but these bacterial factors are not the focus of this review.

In this present minireview, we will discuss known host factors that actively inhibit *H. pylori*-induced inflammation and thereby protect the majority of those infected from gastric cancer and peptic ulcer disease. The complex inflammatory response provides multiple stages that can be regulated by the host. Specifically, the *H. pylori*-infected individual has evolved to coexist with this pathogen by developing a number of features that include *1*) a mucosal barrier that restricts access of *H. pylori* to the epithelial surface, as well as factors that reduce activation of *2*) the innate and *3*) the adaptive immune response to this infection. These are further discussed below and summarized in Table 1.

The Gastric Mucosal Barrier

Gastric mucus is composed of two main overlapping layers of secreted mucins. The outer mucus layer contains mucin 5AC (MUC5AC), which is secreted by surface epithelial mucous cells, while the inner mucus layer predominantly comprises MUC6, which is secreted by mucous neck cells located in the gastric pits. The majority of *H. pylori* are located within the MUC5AC-containing outer mucus layer and are rarely observed within the MUC6-containing layer that lies closest to the epithelial surface (20); only a few of these bacteria contact the epithelial surface. This preferential distribution of *H. pylori* is most likely due to the presence of a number of bactericidal agents within the inner mucus layer. MUC6 has a terminal cap of α-1,4 *N*-acetylglucosamine with antibacterial activity that has been shown to inhibit *H. pylori* growth (25). Given the large quantity of MUC6 present in the inner mucus layer, the antibacterial activity of this mucin is likely to be the main

explanation for the low levels of *H. pylori* in this region, but other bactericidal agents including antimicrobial peptides like β-defensins-2 (40), cathelicidin LL-37 (66), and galectin-3 (43) might also play a role (Fig. 1*A*). In summary, due to these bactericidal properties, the inner mucus layer effectively limits the number of *H. pylori* that reach the gastric epithelium, thereby restricting activation of innate immune receptors on the epithelial surface.

Host Inhibition of the Innate Immune Response to H. pylori

Bacteria that do reach the epithelial surface activate a range of innate immune responses. A number of innate receptors are linked with *H. pylori*-driven gastritis, but perhaps the most important include TLR2, TLR4, and TLR9, the nucleotide binding and oligomerization domain 1 (NOD1), and the NOD-like receptor pyrin domain-containing 3 (NLRP3) receptor (51). Activation of these receptors leads to the induction of intracellular signaling cascades, the majority of which are proinflammatory and normally aimed at clearing the infection. These pathways induce the secretion of cytokines and chemokines (including IL-8, IL-1β, IL-6, IL-12, TNFα) as well as reactive oxygen and nitrogen species, and cyclooxygenase-2. In the case of *H. pylori*, however, this innate inflammatory immune response fails to clear the infection, and if left unregulated the sustained and unrestricted inflammation would likely result in disease. Probably for this reason, the host has developed several mechanisms, discussed hereafter, that dampen the innate, inflammatory immune response to *H. pylori*.

Trefoil factors. Trefoil factors (TFF) are a family of small (7–12 kDa) protease-resistant proteins that are copiously produced by mucus-secreting cells and that protect mucosal surfaces by promoting epithelial repair and healing (59). Two members of this family, TFF1 and TFF2, are progressively downregulated during human gastric cancer development, consistent with both having roles in limiting inflammation (32), a suggestion supported by animal studies whereby mice deficient in either TFF1 or TFF2 were demonstrated to display more severe gastritis after *Helicobacter* infection (29, 58).

TFF1 is found in association with the MUC5AC mucus layer in the stomach and has also been shown to bind *H. pylori* (10), while TFF2 is located within the MUC6 layer (42). TFF2 also appears to have a biologically relevant role in leukocytes, at least in rodents, as *Tff2^{-/-}* leukocytes in isolation exhibit increased inflammatory activity (29). Interestingly, TFF2 ap-

Table 1. *The role of major host factors in regulating* H. pylori-*induced inflammation*

| Factor	Function	Key References
Mucin6	Antibacterial	25
Human β defensin-2	Antibacterial	40
Cathelicidin LL-37	Antibacterial	66
Galectin-3	Antibacterial	43
Trefoil factor-1	Suppression of TNFR1/NF-κB signaling pathway	57, 58
Trefoil factor-2	Negative regulation of IL-1R/NF-κB signaling pathway	16, 29
Gastrokine 2	Mechanism unclear	37
	Possibly via modulation of NF-κB activation	
Mucin1	Limiting *H. pylori* colonization on epithelial surface	6, 36, 39, 61
	Restricting activation of TLR/NF-κB pathway to supress IL-1β production	
Peroxisome proliferator-activated receptor-γ	Inhibition of NF-κB activation	18, 46, 55
Protease activated receptor-1	Restricts activation of interferon regulatory factor 5 to limit induction of Th1 and Th17 responses	9, 62
Regulatory T cells	Suppression of Th1 and Th17 responses mediated via production of IL-10	30, 47–49
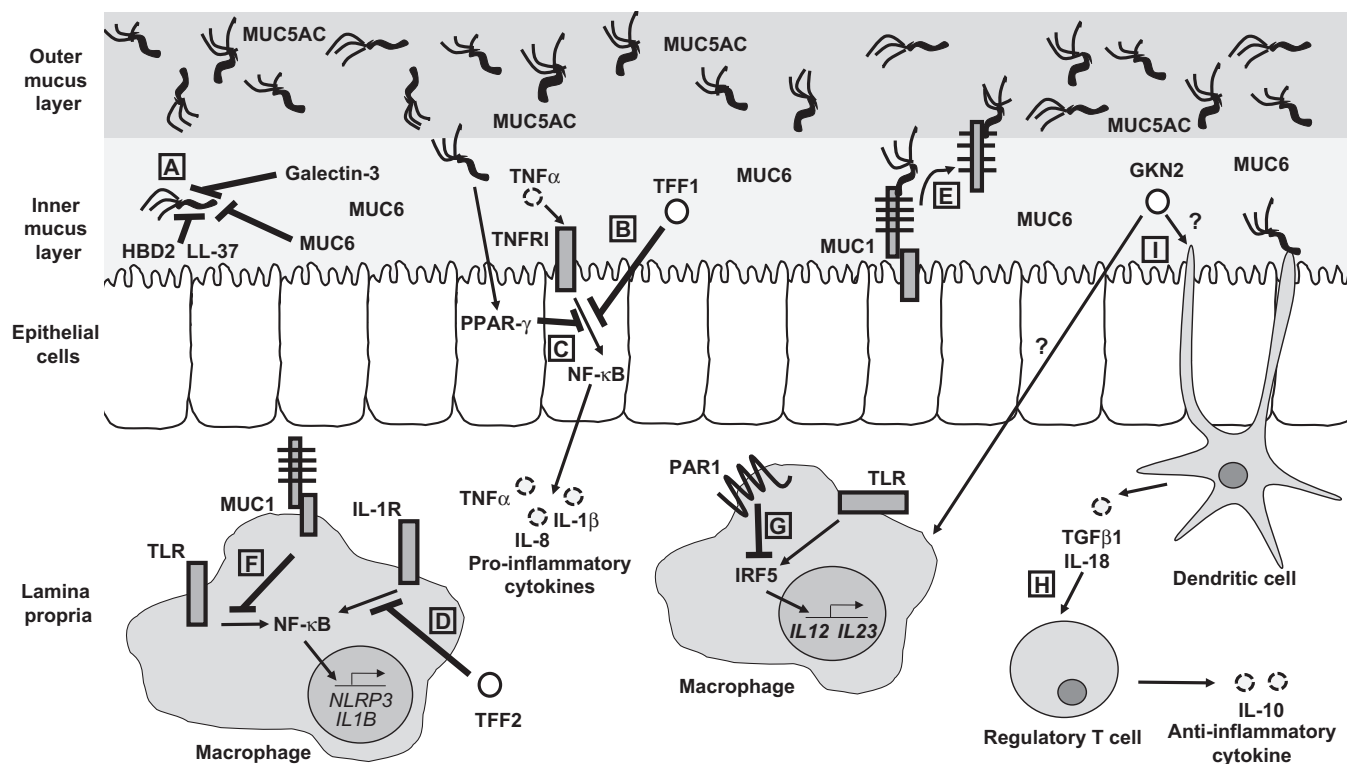
Fig. 1. Host factors that suppress *Helicobacter pylori*-induced inflammation. *A*: the inner mucus layer contains several bactericidal agents including MUC6, cathelicidin LL-37, human β defensin-2, and galectin-3 that can limit *H. pylori* growth, thereby reducing the activation of innate immune receptors on the epithelial surface. *B*: trefoil factor 1 (TFF1) restricts tumor necrosis factor receptor 1 (TNFR1) signaling, which, like peroxisome proliferator-activated receptor-γ (PPAR-γ) (*C*), suppresses the activation of nuclear transcription factor-κB (NF-κB), thus downregulating the production of proinflammatory cytokines. *D*: TFF2 is a negative regulator of IL-1R signaling cascade in macrophages. MUC1 on the apical epithelial surface limits *H. pylori* binding to epithelium by acting as a releasable decoy (*E*), while MUC1 in immune cells (*F*) it suppresses IL-1β production by restricting activation of the TLR/NF-κB pathway and the NLRP3 inflammasome. *G*: protease-activated receptor 1 (PAR1) on immune cells negatively regulates interferon regulatory factor-5 (IRF5) in macrophages, suppressing transcription of the Th1- and Th17-inducing cytokines IL-12 and IL-23. *H*: *H. pylori*-activated dendritic cells produce IL-18 and TGFβ1 that promote the development of regulatory T cells, which also inhibit the Th1 and Th17 responses, although via production of immunosuppressive IL-10. *I*: GKN2 is a potent suppressor of *H. pylori*-induced gastritis, but the mechanism is yet to be elucidated.

pears to have a role in sustaining TFF1 expression (45), which could mean that TFF2 partially mediates its anti-inflammatory effects through TFF1, although suppressive mechanism(s) distinct from TFF1 have been identified.

Multiple innate cellular receptors and subsequent signaling pathways result in activation of nuclear transcription factor-κB (NF-κB; a "master" transcription factor that plays a major role in the innate immune response), including TLRs and receptors for IL-1 (IL-1R) and TNFα (TNFR) (44). Mechanistically, both TFF1 and TFF2 have been inferred to negatively regulate activation of NF-κB. TFF1 inhibits activation of TNFR1 by preventing signaling intermediates, specifically TRAF2, from binding and thus conducting the signaling cascade (57) (Fig. 1*B*). It remains possible that TFF1 might also inhibit NF-κB activation via other pathways.

TFF2 also appears to inhibit NF-κB activation, as *Tff2*$^{-/-}$ cells produce elevated amounts of NF-κB-driven cytokines after stimulation (29). This appears distinct from TFF1 regulation as it is primarily due to suppression of IL-1R signaling (29) (Fig. 1*D*). However, TFF2 can also inhibit certain aspects of TLR4 signaling, as inducible nitric oxide synthase (iNOS) production in response to TLR4 activation of the RAW264.7 macrophage cell line was suppressed by TFF2 treatment (16).

Gastrokines. Gastrokines (GKNs) are small proteins that are almost exclusively expressed in the stomach. Multiple studies

have demonstrated a reduction or loss of expression of GKN1 and GKN2 in gastric cancer, as well as in *H. pylori*-infected tissue (reviewed in Ref. 37). While there is some in vitro evidence for an effect of GKN1 on cells, it is currently unclear whether these effects translate to a meaningful effect in vivo, as GKN1-deficient animals are not available. Hence this will not be discussed here.

Recently, *Gkn2*$^{-/-}$ mice were described and convincingly demonstrated to have an accelerated inflammatory phenotype in response to *H. pylori* infection and in genetic models of gastric cancer (38). The immunophenotype of *Gkn2*$^{-/-}$ epithelial cells was described to be similar to that of wild-type cells (38). However, interestingly, the immune cells present in *Gkn2*$^{-/-}$ stomachs display elevated levels of proinflammatory cytokines (TNFα, IL-1α, IL-6, CXCL1, CCL4) and anti-inflammatory IL-10 (which probably prevents spontaneous gastritis), suggesting that GKN2 has an exogenous effect on resident immune cells (Fig. 1*I*). A separate study has suggested that GKN2, when overexpressed, might suppress NF-κB activation (67), which could be one mechanism by which GKN2 mediates in vivo effects. However, the mechanism of immunomodulation in vivo remains to be determined.

In summary, GKN2 is clearly an important regulatory host factor during *H. pylori* infection. Given this suppressive role, it is likely that GKN2 and possibly GKN1 act as rheostats to

inhibit inflammation during the basal state and, when downregulated as observed during infection or cancer, allow inflammatory responses to pathogens such as *H. pylori*.

Mucin 1. MUC1 is a cell membrane-associated mucin, expressed on the majority of mucosal epithelial cells, as well as most immune cells. Like all mucins, MUC1 contains an extracellular VNTR (variable number of tandem repeats) region, which produces a high degree of polymorphism in molecule length. Epidemiological studies found that short *MUC1* alleles were associated with an increased susceptibility to both gastric cancer (6) and chronic *H. pylori* gastritis (61). This established MUC1 as a potential protective host factor in *H. pylori* pathogenesis, a possibility confirmed when it was shown that mice lacking MUC1 not only were colonized by increased numbers of *H. pylori* but also developed a considerably more severe gastritis (36). Interestingly, these two observations were subsequently found to be due to two quite separate activities of MUC1.

First, the ability of MUC1 to reduce *H. pylori* colonization was shown to be related to epithelial MUC1 acting as a releasable decoy (33) (Fig. 1*E*). Specifically, *H. pylori* binding to MUC1 on epithelial cells triggers the release of the extracellular mucin domain, effectively shedding the bacteria from the mucosal surface, thereby limiting its ability to adhere to the gastric epithelium.

In contrast, however, it was recently shown that, surprisingly, it is MUC1 expressed by immune cells that reduces the severity of *H. pylori*-induced gastritis (39). In particular, MUC1 suppresses IL-1β secretion by macrophages in response to *H. pylori*, and by doing so protects against the development of atrophic gastritis, an important step on the path to gastric cancer (39). A key stage of IL-1β production involves activation of inflammasomes, multiprotein complexes that, when activated, produce active caspase-1 that converts zymogenic pro-IL-1β (as well as pro-IL-18) into active cytokine. While a number of inflammasome complexes are known, IL-1β secretion in response to *H. pylori* occurs via the NLRP3 inflammasome (53), an inflammasome unique in its requirement for NF-κB activation. MUC1 was shown to attenuate the NLRP3 inflammasome by suppressing activation of TLR signaling at the initial steps (before IRAK4, the first kinase in the TLR pathway) (Fig. 1*F*) (39).

Thus MUC1 is a key host factor that can both restrict *H. pylori* attachment to epithelial cells and prevent activation of the NLRP3 inflammasome in immune cells, thereby inhibiting activation of innate immunity via two distinct mechanisms.

Peroxisome proliferator-activated receptor-γ. Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors that belong to the nuclear hormone receptor family. Once activated, PPAR translocates to the nucleus, where it binds to a particular DNA motif (the peroxisome proliferator responsive element, PPRE) of target genes, upregulating their expression. The PPAR family comprise of three subtypes (PPAR-α, β, and γ), of which PPAR-γ has been most studied during *H. pylori* infection.

Evidence suggests a role for PPAR-γ in *H. pylori* infection in humans. PPAR-γ is upregulated in gastric biopsies from *H. pylori*-infected individuals and levels significantly drop after antibiotic eradication (26). Furthermore, a polymorphism in the *PPARG* gene that results in reduced binding of PPAR-γ to PPRE is significantly associated with gastric adenocarcinoma

in *H. pylori*-positive patients (46). Experimentally, treatment with a synthetic PPAR-γ ligand in a rat model of acute gastric inflammation reduces gastritis severity (55), and *Pparg*$^{+/-}$ mice develop more tumors and succumbed to cancer death ahead of wild-type controls (34), demonstrating that PPAR-γ also has anti-tumorigenic activity.

The mechanism by which PPAR-γ limits *H. pylori* pathology is unclear, although treatment of human gastric epithelial cells with a PPAR-γ agonist attenuates *H. pylori*-induced NF-κB activation (18) (Fig. 1*C*), providing at least one mechanism via which PPAR-γ could suppress gastritis.

Host Inhibition of the Adaptive Immune Response to H. pylori

After the innate response to *H. pylori* is initiated, chronic gastritis is perpetuated by establishment of a mixed Th1- and Th17-type proinflammatory adaptive immune response. The host has also developed a number of mechanisms that, in most individuals, limit this adaptive response.

Protease activated receptor-1. Pathogenic infection and ensuing inflammation commonly result in the release of proteases. These extracellular proteases can act as warning signals detected by protease-activated receptors (PAR), G protein-coupled receptors expressed by a wide range of cells including epithelial and immune cells. PAR activation, which occurs following exposure to an appropriate, typically serine, protease induces cell signaling events with a range of potential effects including cell proliferation and cytokine secretion (56).

One such receptor, PAR1, has a potent role in protecting against *H. pylori*-induced gastritis. Mice lacking PAR1 develop a markedly more severe *H. pylori*-induced atrophic gastritis (62), and a polymorphism in PAR1 (the *PAR1* IVSn-14 *T* allele) has been identified as a risk factor for *H. pylori*-associated gastric cancer in humans (7). As the *PAR1* IVSn-14 *T* allele variant is linked with reduced PAR1 expression (14), the association of this polymorphism with gastric cancer susceptibility may have resulted from decreased gastric PAR1 leading to a reduced ability to control excessive inflammation.

PAR1 potentially inhibits *H. pylori*-induced gastritis by effects on both epithelial and immune cells. PAR1 reduces the MIP-2α (a functional homologue of human IL-8) response of mouse gastric epithelial cells to *H. pylori* stimulation, which would be expected to limit neutrophil recruitment into the infected gastric mucosa (62). However, bone marrow chimera studies reveal that the dominant protective effect is mediated by PAR1-expressing immune cells (9). In particular, PAR1 is a potent suppressor of interferon regulatory factor 5 (IRF5) in macrophages (9) (Fig. 1*G*). IRF5 is an important transcription factor involved in IL-12 and IL-23 secretion, key drivers of Th1 and Th17 acquired immune responses, respectively (28). PAR1 activation prevented IRF5 induction in macrophages exposed to *H. pylori* with downstream effects on IFNγ and IL-17 responses in vivo (9). Hence PAR1 appears to protect against *H. pylori*-induced gastritis by regulating IRF5, thereby restricting both Th1 and Th17 adaptive responses and limiting the development of severe gastric pathologies.

Regulatory T cells. Regulatory T cells (Tregs) are potentially the most important and certainly the best studied suppressors of *H. pylori*-induced inflammation. Tregs are

typically $CD4^+CD25^+FOXP3^+$ cells that can be broadly divided into natural Tregs (nTregs) and inducible Tregs (iTregs), the former being nonspecific and developing in the thymus, while the latter develop from naive T cells under certain circumstances (50). Typically, nTregs suppress other T cells via direct contact, in contrast to iTregs, which typically suppress other cells by secreting regulatory cytokines including IL-10 (50).

Multiple studies have demonstrated the importance of Tregs in regulating *H. pylori* pathology. Tregs numbers increase in *H. pylori*-infected patients, with an inadequate Treg response associated with increased disease severity (49). Primarily, these Tregs are recruited via the CCL20 chemokine, secreted by epithelial cells in response to *H. pylori* (11). Consistent with these human data, gastric *Foxp3* mRNA levels increase following *H. pylori* infection of mice (47) and $Foxp3^+$ Tregs develop in draining lymph nodes (41). Moreover, the adoptive transfer of splenocytes into T cell-deficient mice resulted in a more severe *H. pylori*-induced gastritis if the cells were Treg depleted (48), as did Treg depletion of conventional mice by the injection of an anti-CD25 antibody prior to *H. pylori* infection (47), demonstrating a clear role for Tregs in suppressing *H. pylori* pathology.

The main mechanism by which Tregs reduces gastritis severity is most likely by secreting the potent immunosuppressive cytokine IL-10 (Fig. 1*H*). In mice, particularly severe gastritis is observed in $Il10^{-/-}$ animals after infection (3, 8) and $Il10^{-/-}$ Tregs are largely incapable of suppressing *H. pylori* gastritis (30). IL-10 has also been shown to increase in humans during gastritis (5) and an association between a low IL-10-producing haplotype variation has been found with atrophic gastritis (15).

During *H. pylori* infection, several cytokines promote Treg development, including the pleiotropic cytokine TGFβ1 and IL-18 (Fig. 1*H*). While TGFβ1 is an important driver of Treg development, it can also exert direct immunosuppressive activity on a variety of cells (31). TGFβ1 derived from both epithelial and dendritic cells can promote Treg development in response to *H. pylori* (4, 24). A *TGFB1* promoter SNP ($-509C>T$) that results in increased TGFβ1 production is negatively associated with gastric cancer in humans (23), demonstrating the potentially important protective role of this cytokine in humans.

IL-18, which matures via an inflammasome in a similar fashion to IL-1β, is another important promoter of Treg development during *H. pylori* infection (41). Mice that lack IL-18 develop more severe *H. pylori*-induced gastritis (21), and it has been shown that it is specifically IL-18 from dendritic cells that is essential for *H. pylori* induction of protective Tregs (41).

Conclusion

This review discusses host factors for which there is clear evidence of a role in restricting the severity of *H. pylori*-induced gastritis. Such factors have evolved to ensure the inflammatory response to this pathogen is regulated and disease does not ensue; in most cases this is successful. From the literature presented here, it is evident that most known innate regulators suppress inflammation primarily by restricting activation of NF-κB, while Tregs are a key negative regulator of the adaptive immune response to *H. pylori* infection. There are

likely more protective host factors that contribute to this regulation; some possibilities include myeloid-derived suppressor cells (12), regulatory B cells (52), matrix metalloproteinase-7 (27), calgranulin C (19), and microRNA-155 (65), not discussed in this minireview as their roles remain controversial.

Currently, the only treatment option for *H. pylori* infection is antibiotic eradication therapy, but with the rise in antibiotic resistance and recurrence of infection the failure rate of this therapy is increasing (17). Given that it is gastritis and not directly colonization that drives peptic ulcer and gastric cancer development, a feasible approach to managing these diseases in future could be by pharmacologically enhancing host regulatory factors to suppress gastritis. For this, discovering exactly how the host maintains inflammation in most individuals is an important initial step. Additionally, the identification of key gain or loss of function polymorphisms in regulatory host genes could be used clinically to help identify individuals at risk of disease and thus better target gastric cancer and/or peptic ulcer disease treatment strategies.

DISCLOSURES

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AUTHOR CONTRIBUTIONS

P.D. prepared figures; P.D. and P.S. drafted manuscript; P.D., G.Z.N., and P.S. edited and revised manuscript; P.D., G.Z.N., and P.S. approved final version of manuscript.

REFERENCES

1. **Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S.** Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science* 300: 1430–1434, 2003.
2. **Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, Brooks EG, Graham DY, Reyes VE, Ernst PB.** Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. *Gastroenterology* 114: 482–492, 1998.
3. **Berg DJ, Lynch NA, Lynch RG, Lauricella DM.** Rapid development of severe hyperplastic gastritis with gastric epithelial dedifferentiation in *Helicobacter felis*-infected IL-10$^{-/-}$ mice. *Am J Pathol* 152: 1377, 1998.
4. **Beswick EJ, Pinchuk IV, Earley RB, Schmitt DA, Reyes VE.** Role of gastric epithelial cell-derived transforming growth factor beta in reduced CD4+ T cell proliferation and development of regulatory T cells during *Helicobacter pylori* infection. *Infect Immun* 79: 2737–2745, 2011.
5. **Bodger K, Wyatt JI, Heatley RV.** Gastric mucosal secretion of interleukin-10: relations to histopathology, *Helicobacter pylori* status, and tumour necrosis factor-alpha secretion. *Gut* 40: 739–744, 1997.
6. **Carvalho F, Seruca R, David L, Amorim A, Seixas M, Bennett E, Clausen H, Sobrinho-Simoes M.** *MUC1* gene polymorphism and gastric cancer—an epidemiological study. *Glycoconj J* 14: 107–111, 1997.
7. **Castano-Rodriguez N, Kaakoush NO, Goh KL, Fock KM, Chionh YT, Sutton P, Mitchell HMI.** PAR-1 polymorphisms and risk of *Helicobacter pylori*-related gastric cancer in a Chinese population. *Anticancer Res* 32: 3715–3721, 2012.
8. **Chen W, Shu D, Chadwick VS.** *Helicobacter pylori* infection: mechanism of colonization and functional dyspepsia reduced colonization of gastric mucosa by *Helicobacter pylori* in mice deficient in interleukin-10. *J Gastroenterol Hepatol* 16: 377–383, 2001.
9. **Chionh YT, Ng GZ, Ong L, Arulmuruganar A, Stent A, Saeed MA, Wee JL, Sutton P.** Protease-activated receptor 1 suppresses *Helicobacter*

pylori gastritis via the inhibition of macrophage cytokine secretion and interferon regulatory factor 5. *Mucosal Immunol* 8: 68–79, 2015.

10. **Clyne M, Dillon P, Daly S, O'Kennedy R, May FE, Westley BR, Drumm B.** *Helicobacter pylori* interacts with the human single-domain trefoil protein TFF1. *Proc Natl Acad Sci USA* 101: 7409–7414, 2004.

11. **Cook KW, Letley DP, Ingram RJ, Staples E, Skjoldmose H, Atherton JC, Robinson K.** CCL20/CCR6-mediated migration of regulatory T cells to the *Helicobacter pylori*-infected human gastric mucosa. *Gut* 63: 1550–1559, 2014.

12. **Dolcetti L, Marigo I, Mantelli B, Peranzoni E, Zanovello P, Bronte V.** Myeloid-derived suppressor cell role in tumor-related inflammation. *Cancer Lett* 267: 216–225, 2008.

13. **Dooley CP, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, Blaser MJ.** Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 321: 1562–1566, 1989.

14. **Dupont A, Fontana P, Bachelot-Loza C, Reny JL, Bieche I, Desvard F, Aiach M, Gaussem P.** An intronic polymorphism in the PAR-1 gene is associated with platelet receptor density and the response to SFLLRN. *Blood* 101: 1833–1840, 2003.

15. **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* 124: 1193–1201, 2003.

16. **Giraud AS, Pereira PM, Thim L, Parker LM, Judd LM.** TFF-2 inhibits iNOS/NO in monocytes, and nitrated protein in healing colon after colitis. *Peptides* 25: 803–809, 2004.

17. **Graham DY, Fischbach L.** *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. *Gut* 59: 1143–1153, 2010.

18. **Gupta RA, Polk DB, Krishna U, Israel DA, Yan F, DuBois RN, Peek RM.** Activation of peroxisome proliferator-activated receptor γ suppresses nuclear factor κB-mediated apoptosis induced by *Helicobacter pylori* in gastric epithelial cells. *J Biol Chem* 276: 31059–31066, 2001.

19. **Haley KP, Delgado AG, Piazuelo MB, Mortensen BL, Correa P, Damo SM, Chazin WJ, Skaar EP, Gaddy JA.** The human antimicrobial protein calgranulin C participates in control of *Helicobacter pylori* growth and regulation of virulence. *Infect Immun* 83: 2944–2956, 2015.

20. **Hidaka E, Ota H, Hidaka H, Hayama M, Matsuzawa K, Akamatsu T, Nakayama J, Katsuyama T.** *Helicobacter pylori* and two ultrastructurally distinct layers of gastric mucous cell mucins in the surface mucous gel layer. *Gut* 49: 474–480, 2001.

21. **Hitzler I, Sayi A, Kohler E, Engler DB, Koch KN, Hardt WD, Muller A.** Caspase-1 has both proinflammatory and regulatory properties in *Helicobacter* infections, which are differentially mediated by its substrates IL-1beta and IL-18. *J Immunol* 188: 3594–3602, 2012.

22. **International Agency for Research on Cancer.** GLOBOCAN 2012: Estimated cancer incidence, mortality and prevalence worldwide in 2012. World Health Organization. http://globocan.iarc.fr/Default.aspx.

23. **Jin G, Wang L, Chen W, Hu Z, Zhou Y, Tan Y, Wang J, Hua Z, Ding W, Shen J, Zhang Z, Wang X, Xu Y, Shen H.** Variant alleles of TGFB1 and TGFBR2 are associated with a decreased risk of gastric cancer in a Chinese population. *Int J Cancer* 120: 1330–1335, 2007.

24. **Kao JY, Zhang M, Miller MJ, Mills JC, Wang B, Liu M, Eaton KA, Zou W, Berndt BE, Cole TS, Takeuchi T, Owyang SY, Luther J.** *Helicobacter pylori* immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. *Gastroenterology* 138: 1046–1054, 2010.

25. **Kawakubo M, Ito Y, Okimura Y, Kobayashi M, Sakura K, Kasama S, Fukuda MN, Fukuda M, Katsuyama T, Nakayama J.** Natural antibiotic function of a human gastric mucin against *Helicobacter pylori* infection. *Science* 305: 1003–1006, 2004.

26. **Konturek PC, Kania J, Kukharsky V, Raithel M, Ocker M, Rembiasz K, Hahn EG, Konturek SJ.** Implication of peroxisome proliferator-activated receptor-gamma and proinflammatory cytokines in gastric carcinogenesis: Link to *Helicobacter pylori* infection. *J Pharm Sci* 96: 134–143, 2004.

27. **Krakowiak MS, Noto JM, Piazuelo MB, Hardbower DM, Romero-Gallo J, Delgado A, Chaturvedi R, Correa P, Wilson KT, Peek RM.** Matrix metalloproteinase 7 restrains *Helicobacter pylori*-induced gastric inflammation and premalignant lesions in the stomach by altering macrophage polarization. *Oncogene* 34: 1865–1871, 2015.

28. **Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, Hussell T, Feldmann M, Udalova IA.** IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* 12: 231–238, 2011.

29. **Kurt-Jones EA, Cao L, Sandor F, Rogers AB, Whary MT, Nambiar PR, Cerny A, Bowen G, Yan J, Takaishi S, Chi AL, Reed G, Houghton J, Fox JG, Wang TC.** Trefoil family factor 2 is expressed in murine gastric and immune cells and controls both gastrointestinal inflammation and systemic immune responses. *Infect Immun* 75: 471–480, 2007.

30. **Lee CW, Rao VP, Rogers AB, Ge Z, Erdman SE, Whary MT, Fox JG.** Wild-type and interleukin-10-deficient regulatory T cells reduce effector T-cell mediated gastroduodenitis in *Rag2*$^{-/-}$ mice, but only wild-type regulatory T cells suppress *Helicobacter pylori* gastritis. *Infect Immun* 75: 2699–2707, 2007.

31. **Letterio JJ, Roberts AB.** Regulation of immune responses by TGF-beta. *Annu Rev Immunol* 16: 137–161, 1998.

32. **Leung WK, Yu J, Chan FK, To KF, Chan MW, Ebert MP, Ng EK, Chung SC, Malfertheiner P, Sung JJ.** Expression of trefoil peptides (TFF1, TFF2, and TFF3) in gastric carcinomas, intestinal metaplasia, and non-neoplastic gastric tissues. *J Pathol* 197: 582–588, 2002.

33. **Linden SK, Sheng YH, Every AL, Miles KM, Skoog EC, Florin THJ, Sutton P, McGuckin MA.** MUC1 limits *Helicobacter pylori* infection both by steric hindrance and by acting as a releasable decoy. *PLoS Pathog* 5: 2009.

34. **Lu J, Imamura K, Nomura S, Mafune Ki Nakajima A, Kadowaki T, Kubota N, Terauchi Y, Ishii G, Ochiai A, Esumi H, Kaminishi M.** Chemopreventive effect of peroxisome proliferator-activated receptor γ on gastric carcinogenesis in mice. *Cancer Res* 65: 4769–4774, 2005.

35. **Malfertheiner P, Chan FKL, McColl KEL.** Peptic ulcer disease. *Lancet* 374: 1449–1461.

36. **McGuckin MA, Every AL, Skene CD, Linden SK, Chionh YT, Swierczak A, McAuley J, Harbour S, Kaparakis M, Ferrero R, Sutton P.** Muc1 mucin limits both *Helicobacter pylori* colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology* 133: 1210–1218, 2007.

37. **Menheniott TR, Kurklu B, Giraud AS.** Gastrokines: stomach-specific proteins with putative homeostatic and tumor suppressor roles. *Am J Physiol Gastrointest Liver Physiol* 304: G109–G121, 2013.

38. **Menheniott TR, O'Connor L, Chionh YT, Däbritz J, Scurr M, Rollo BN, Ng GZ, Jacobs S, Catubig A, Kurklu B, Mercer S, Minamoto T, Ong DE, Ferrero RL, Fox JG, Wang TC, Sutton P, Judd LM, Giraud AS.** Loss of gastrokine-2 drives premalignant gastric inflammation and tumor progression. *J Clin Invest* 126: 1383–1400.

39. **Ng GZ, Menheniott TR, Every AL, Stent A, Judd LM, Chionh YT, Dhar P, Komen JC, Giraud AS, Wang TC, McGuckin MA, Sutton P.** The MUC1 mucin protects against *Helicobacter pylori* pathogenesis in mice by regulation of the NLRP3 inflammasome. *Gut* 65: 1087–1099, 2016.

40. **O'Neil DA, Cole SP, Martin-Porter E, Housley MP, Liu L, Ganz T, Kagnoff MF.** Regulation of human beta-defensins by gastric epithelial cells in response to infection with *Helicobacter pylori* or stimulation with interleukin-1. *Infect Immun* 68: 5412–5415, 2000.

41. **Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, Maxeiner J, Hansson M, Taube C, Quiding-Järbrink M, Müller A.** DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*-specific immune tolerance, and asthma protection. *J Clin Invest* 122: 1082–1096, 2012.

42. **Ota H, Hayama M, Momose M, El-Zimaity HM, Matsuda K, Sano K, Maruta F, Okumura N, Katsuyama T.** Co-localization of TFF2 with gland mucous cell mucin in gastric mucous cells and in extracellular mucous gel adherent to normal and damaged gastric mucosa. *Histochem Cell Biol* 126: 617–625, 2006.

43. **Park AM, Hagiwara S, Hsu DK, Liu FT, Yoshie O.** Galectin-3 plays an important role in innate immunity to gastric infection by *Helicobacter pylori*. *Infect Immun* 84: 1184–1193, 2016.

44. **Perkins ND.** Integrating cell-signalling pathways with NF-kB and IKK function. *Nat Rev Mol Cell Biol* 8: 49–62, 2007.

45. **Peterson AJ, Menheniott TR, O'Connor L, Walduck AK, Fox JG, Kawakami K, Minamoto T, Ong EK, Wang TC, Judd LM, Giraud AS.** *Helicobacter pylori* infection promotes methylation and silencing of trefoil factor 2, leading to gastric tumor development in mice and humans. *Gastroenterology* 139: 2005–2017, 2010.

46. **Prasad KN, Saxena A, Ghoshal UC, Bhagat MR, Krishnani N.** Analysis of Pro12Ala PPAR gamma polymorphism and *Helicobacter pylori* infection in gastric adenocarcinoma and peptic ulcer disease. *Ann Oncol* 19: 1299–1303, 2008.

47. **Rad R, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP, Reindl W, Dossumbekova A, Friedrich M, Saur D, Wagner H, Schmid RM, Prinz C.** CD25+/Foxp3+ T cells regulate gastric inflammation and *Helicobacter pylori* colonization in vivo. *Gastroenterology* 131: 525–537, 2006.

48. **Raghavan S, Fredriksson M, Svennerholm AM, Holmgren J, Suri-Payer E.** Absence of CD4(+)CD25(+) regulatory T cells is associated with a loss of regulation leading to increased pathology in Helicobacter pylori-infected mice. *Clin Exp Immunol* 132: 393–400, 2003.

49. **Robinson K, Kenefeck R, Pidgeon EL, Shakib S, Patel S, Polson RJ, Zaitoun AM, Atherton JC.** *Helicobacter pylori*-induced peptic ulcer disease is associated with inadequate regulatory T cell responses. *Gut* 57: 1375–1385, 2008.

50. **Sakaguchi S, Yamaguchi T, Nomura T, Ono M.** Regulatory T cells and immune tolerance. *Cell* 133: 775–787, 2008.

51. **Salama NR, Hartung ML, Mueller A.** Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. *Nat Rev Microbiol* 11: 385–399, 2013.

52. **Sayi A, Kohler E, Toller IM, Flavell RA, Müller W, Roers A, Müller A.** TLR-2-activated B cells suppress *Helicobacter*-induced preneoplastic gastric immunopathology by inducing T regulatory-1 cells. *J Immunol* 186: 878–890, 2011.

53. **Semper RP, Mejias-Luque R, Gross C, Anderl F, Muller A, Vieth M, Busch DH, Prazeres da Costa C, Ruland J, Gross O, Gerhard M.** *Helicobacter pylori*-induced IL-1beta secretion in innate immune cells is regulated by the NLRP3 inflammasome and requires the cag pathogenicity island. *J Immunol* 193: 3566–3576, 2014.

54. **Shiomi S, Toriie A, Imamura S, Konishi H, Mitsufuji S, Iwakura Y, Yamaoka Y, Ota H, Yamamoto T, Imanishi J, Kita M.** IL-17 is involved in *Helicobacter pylori*-induced gastric inflammatory responses in a mouse model. *Helicobacter* 13: 518–524, 2008.

55. **Slomiany BL, Slomiany A.** Suppression of gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide by peroxisome proliferator-activated receptor γ activation. *IUBMB Life* 53: 303–308, 2002.

56. **Soh UJ, Dores MR, Chen B, Trejo J.** Signal transduction by protease-activated receptors. *Br J Pharmacol* 160: 191–203, 2010.

57. **Soutto M, Belkhiri A, Piazuelo MB, Schneider BG, Peng D, Jiang A, Washington MK, Kokoye Y, Crowe SE, Zaika A, Correa P, Peek RM, El-Rifai W.** Loss of TFF1 is associated with activation of NF-κB-mediated inflammation and gastric neoplasia in mice and humans. *J Clin Invest* 121: 1753–1767, 2011.

58. **Soutto M, Chen Z, Katsha AM, Romero-Gallo J, Krishna US, Piazuelo MB, Washington MK, Peek RM, Belkhiri A, El-Rifai WM.** Trefoil factor 1 expression suppresses *Helicobacter pylori*-induced inflammation in gastric carcinogenesis. *Cancer* 121: 4348–4358, 2015.

59. **Taupin D, Podolsky DK.** Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol* 4: 721–732, 2003.

60. **Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ.** *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 345: 784–789, 2001.

61. **Vinall LE, King M, Novelli M, Green CA, Daniels G, Hilkens J, Sarner M, Swallow DM.** Altered expression and allelic association of the hypervariable membrane mucin MUC1 in *Helicobacter pylori* gastritis. *Gastroenterology* 123: 41–49, 2002.

62. **Wee JLK, Chionh YT, Ng GZ, Harbour SN, Allison C, Pagel CN, Mackie EJ, Mitchell HM, Ferrero RL, Sutton P.** Protease-activated receptor-1 down-regulates the murine inflammatory and humoral response to *Helicobacter pylori*. *Gastroenterology* 138: 573–582.

63. **Wotherspoon AC, Doglioni C, Diss TC, Pan LX, Moschini A, Deboni M, Isaacson PG.** Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet* 342: 575–577, 1993.

64. **Wroblewski LE, Peek RM, Wilson KT.** *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. *Clin Microbiol Rev* 23: 713–739, 2010.

65. **Xiao B, Liu Z, Li BS, Tang B, Li W, Guo G, Shi Y, Wang F, Wu Y, Tong WD, Guo H, Mao XH, Zou QM.** Induction of microRNA-155 during *Helicobacter pylori* infection and its negative regulatory role in the inflammatory response. *J Infect Dis* 200: 916–925, 2009.

66. **Zhang L, Yu J, Wong CC, Ling TK, Li ZJ, Chan KM, Ren SX, Shen J, Chan RL, Lee CC, Li MS, Cheng AS, To KF, Gallo RL, Sung JJ, Wu WK, Cho CH.** Cathelicidin protects against *Helicobacter pylori* colonization and the associated gastritis in mice. *Gene Ther* 20: 751–760, 2013.

67. **Zhao Z, Wang F, Fang C, Wang G, Shuang J, Chu G, Hu S, Chen L, Du J.** A possible role of GDDR in the development of *Helicobacter pylori*-associated gastric cancer. *Tumour Biol*. 2016 Feb 3. [Epub ahead of print].