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REVIEW

Systems-wide analyses of mucosal immune responses to *Helicobacter pylori* at the interface between pathogenicity and symbiosis

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ABSTRACT

Helicobacter pylori is the dominant member of the gastric microbiota in over half of the human population of which 5–15% develop gastritis or gastric malignancies. Immune responses to *H. pylori* are characterized by mixed T helper cell, cytotoxic T cell and NK cell responses. The presence of Tregs is essential for the control of gastritis and together with regulatory CX3CR1+ mononuclear phagocytes and immune-evasion strategies they enable life-long persistence of *H. pylori*. This *H. pylori*-induced regulatory environment might contribute to its cross-protective effect in inflammatory bowel disease and obesity. Here we review host-microbe interactions, the development of pro- and anti-inflammatory immune responses and how the latter contribute to *H. pylori's* role as beneficial member of the gut microbiota. Furthermore, we present the integration of existing and new data into a computational/mathematical model and its use for the investigation of immunological mechanisms underlying initiation, progression and outcomes of *H. pylori* infection.

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Introduction

H. pylori is a Gram-negative, microaerophilic bacterium within the class of Epsilonproteobacteria. It chronically colonizes the human gastric microenvironment and is one of the most genetically diverse bacterial species that has colonized the stomach since early in human evolution.[1,2] The study of *H. pylori* populations such as hpAfrica, hpEurope, hspEAsia, and hspAmerind[3] has delineated competition between strains and suggests co-adaptation and co-evolution with its host that parallels human migration throughout the globe.

Since its initial discovery by Marshall and Warren,[4] *H. pylori* has been associated with the development of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma.[5,6] Although it is present in the stomach of over 50% of the human population worldwide, only 15% develop serious gastric and duodenal pathologies. Mounting clinical and epidemiological evidence suggests that the presence of *H. pylori* can protect from the development of various diseases including esophageal and cardial pathologies,[7-10] childhood asthma and allergies.[11-14] Originally viewed solely as a pathogen, recent studies are unveiling commensal and symbiotic roles for *H. pylori* as the predominant member of the human gastric microbiota.[15] Therefore, investigating the complex tolerance mechanisms that facilitate the co-existence between *H. pylori* and its human host may yield a deeper understanding of mechanisms of immunoregulation at the mucosal sites. This is a first step toward predicting pathogenic versus beneficial health outcomes of host-*H. pylori* interactions.

The life-long persistence of *H. pylori* in the human stomach suggests that the host response fails to clear the infection and induces an underlying regulatory response. Indeed, *H. pylori* induces a mixed immune response characterized by T helper (Th) 1, Th17 and regulatory T cell (Treg) responses. Whether *H. pylori* exerts a protective effect in the context of a dysregulated immune response or whether it contributes to cell damage and malignant transformation is dependent on host-microbial interactions.

This review sheds new light on the complex interactions of host- and microbial-factors in immune

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responses to *H. pylori* specifically emphasizing the role of inflammasome and TLR signaling in shaping the innate and adaptive T cell responses. We will further discuss novel mechanistic insights leading to *H. pylori*-induced gastritis and mechanisms of immune evasion by *H. pylori* and how this might help explain the role of *H. pylori* as an amphibiont at the interface between commensalism/symbiosis and pathogenicity. Furthermore, we will discuss the use of novel computational modeling approaches to systematically integrate existing knowledge and varied datasets into information processing representations of the mucosal immune system. These computational approaches have facilitated characterizing emerging behaviors and improving our systems-wide understanding of the mechanisms of host-*H. pylori* interactions implicated in the initiation, progression and health outcomes of infection.

H. pylori and its human host: An intimate relationship

H. pylori can persist in the human stomach for a lifetime.[16] Its ability to survive in the gastric niche is tightly connected to the expression of various pathogenicity factors that enable adherence and penetration of the epithelial cell layer and manipulation of innate and adaptive immune responses at the gastric mucosa. Dozens of bacterial factors are involved in *H. pylori*-mediated colonization and molecular pathogenesis including adhesins, outer membrane proteins,[17] urease, catalase, neutrophil-activating protein A (NapA), peptidoglycan (PG), vacuolating cytotoxin A (VacA) and the cag pathogenicity island (*cag*PAI) including cytotoxin-associated gene A (CagA).[18] Figure 1 provides an overview of *H. pylori*'s colonization process and highlights bacterial factors that are important for persistence, survival and the initiation of immune responses. For a detailed description of relevant pathogenicity factors we would like to refer the reader to recent review articles on this topic.[19,20]

Physical contact and the localization of *H. pylori* within in the gastric mucosa are critical determinants for immunopathology. Approximately 20% of *H. pylori* organisms in the stomach adhere to the surface of gastric epithelial cells.[6] This physical contact causes cellular damage to the epithelium, induces inflammation and facilitates the delivery of toxins,[21,22] which in turn promotes bacterial invasion and persistence. Upon CagA injection *H. pylori* exploits the apical epithelial cell surface as a replicative niche.[23] Furthermore, *H. pylori* can establish colonies deep in the gastric glands (Fig. 1). The protein ChePep is necessary for this ability by regulating flagellar rotation through the chemotaxis system,[24] and mutant *H. pylori* strains lacking this chemotaxis ability are excellent tools for forming a comprehensive picture of how microscopic biogeography shapes the type and intensity of mucosal immune responses. Despite its initial classification as extracellular pathogen, recent studies revealed the facultative intracellular nature of *H. pylori*. It invades and multiplies in gastric epithelial cells[25,26] and adult epithelial progenitor cells[27] thereby serving as a repository for *H. pylori* and protecting it from antibiotic eradication. Although the mechanisms of invasion and persistance are not well understood, the importance of *H. pylori* invasin NudA for host cell uptake was reported[28] *in vitro* and dependence on c-Met and the type IV secretion system (T4SS) were determined.[29]

Whether *H. pylori* exerts a protective effect in the context of a dysregulated immune response or whether it contributes to immunopathology, cell damage and malignant transformation is dependent on host-pathogen interactions. The genetic background of both *H. pylori* and the host contribute to disease etiology. On the microbial side the presence of an intact *cag*PAI[30] and the s1m1 variant of VacA among other factors have been associated with more severe pathology and an increased risk for gastroduodenal disease.[31] The importance of the host's immune response in delineating the outcome of chronic *H. pylori* infection is reflected in the association of genetic polymorphisms with a higher risk for developing gastric cancer. These include IL-1β,[32] genes involved in Toll like receptor (TLR) and NOD-like receptor (NLR) signaling pathways[33,34] and the immunoregulatory transciption factor peroxisome proliferator activated receptor γ (PPAR-γ).[35] The relevance of these host factors will become apparent in the following sections discussing the current knowledge on innate and adaptive immune responses to *H. pylori* and how its virulence factors influence these processes.

Initiating the immune response to *H. pylori*

Sensing of pathogen associated molecular patterns (PAMPs) including PG, lipopolysaccharide (LPS),

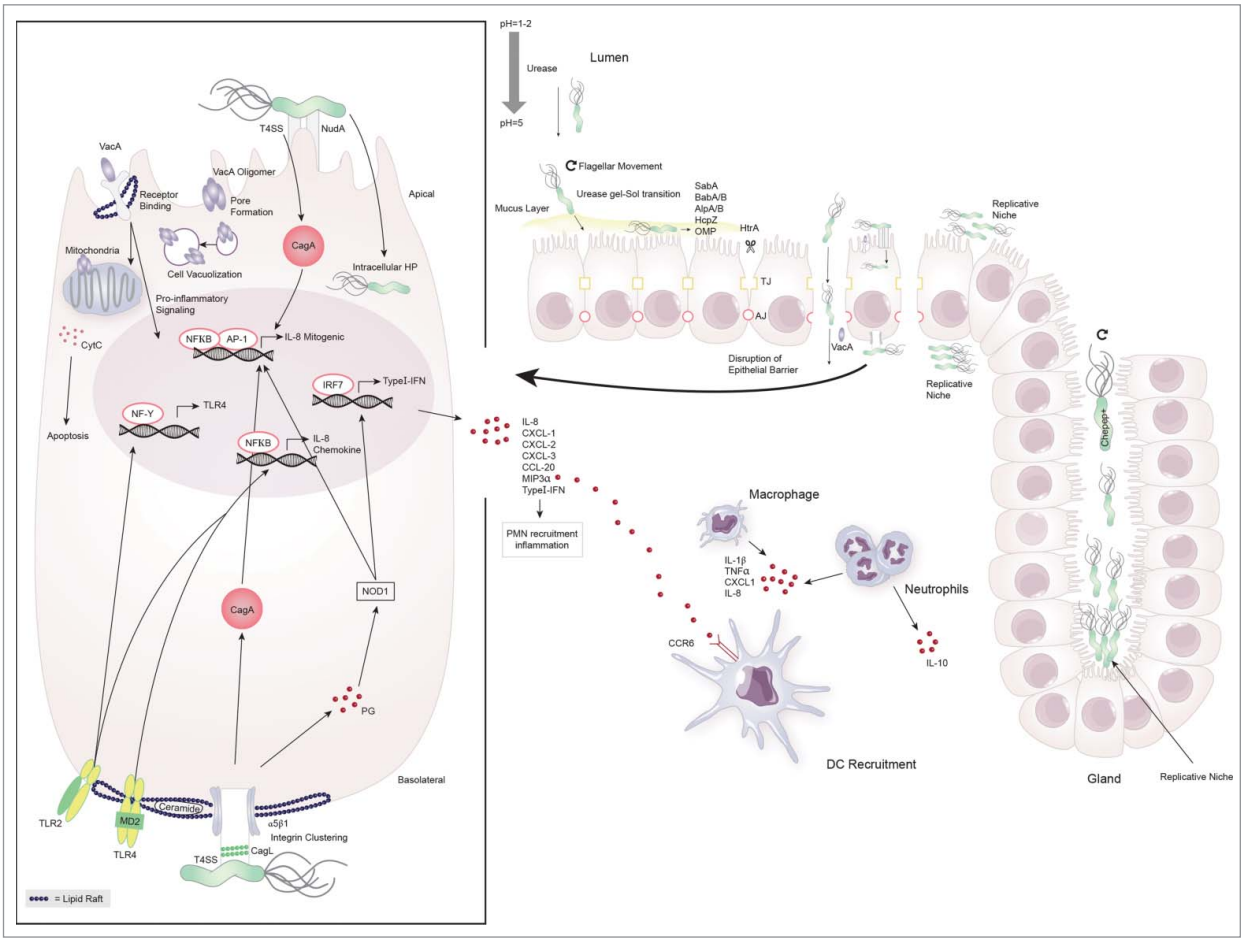

Figure 1. Colonization of the gastric niche and initiation of immune responses by *H. pylori*. *H. pylori* possesses a number of virluence factors that aid in its ability to colonize and exploit the gastric niche for survival and replication. It elevates the gastric pH by secretion of urease and traverses the mucus layer through flagellar movement and urease-induced gel-sol transition. Once in contact with the epithelial cell layer it binds to the apical cell surface using various adhesins (SabA, BabA/B, AlpA/B, HopZ, OMP) causing cell damage and facilitating the delivery of toxins. *H. pylori* disrupts the cell barrier by breaking up tight- and adherent junctions (TJ, AJ) through HtrA and enters the lamina propria to replicate at the basolateral cell surface. *H. pylori* further exploits gastric glands and the apical cell surface as replicative niche for which the proteins Chepep and the virulence factor CagA respectively are essential. At the basolateral cell surface *H. pylori* forms the type 4 secretion system (T4SS) encoded by the cag pathogenicity island and causes integrin clustering at lipid rafts to inject CagA into epithelial cells. Peptidoglycan (PG) leaks through the T4SS and is recognized by the pathogen recognition receptor (PRR) NOD1. These two virulence factors induce the transcription of host-cell genes including IL-8, chemokines and type-I interferons (type-I IFN) through NFκB/AP1 and IRF7, respectively. *H. pylori* further causes host cell remodeling and damage through VacA. This toxin either heterodimerizes and forms pores in the cell membrane or enters by receptor binding to cause cell-vacuolization, pro-inflammatory cytokine signaling and cytochrome C (CytC)- induced apoptosis. Epithelial cells at the mucosal barrier are involved in initiating the immune response to *H. pylori* by antigen-induced TLR-2 and -4 signaling through NFκB. This is further amplified by the induction of TLR-4 transcription via NF-Y-mediated TLR-2 signaling. The subsequent secretion of chemokines attracks peripheral mononuclear cells (PMN) including neutrophils and dendritic cells to the site of infection.

flagellin and unmethylated CpG DNA by pathogen recognition receptors (PRRs) present on epithelial cells, antigen presenting cells and neutrophils is required for the initiation of immune responses to *H. pylori*. Recognition of PAMPs is mediated by membrane-bound TLRs, retinoic acid-inducible gene 1 (RIG-I)-like receptors, cytosolic NLRs and C-type lectins.[36]

The T4SS plays a crucial role in initiating immune responses to *H. pylori*. Upon contact with the host cell it forms a needle-like structure which protrudes from the bacterial surface and delivers virulence factors (e.g. CagA and PG) into the host cell.[37,38] Intracellular PG is specifically recognized by NOD1 and causes the phosphorylation of MAPK, the activation of NF-κB as well as AP-1 and subsequent release of IL-8 by

epithelial cells.[39,40] NOD1-mediated NF-κB activation is dependent on the delivery of PG into the host cell via the T4SS and requires the interaction with host $\alpha_5\beta_1$ integrin localized to lipid rafts of AGS cells.[41] Cholesterol-rich microdomains, also known as lipid rafts, are important sites for receptor complex formation upon *H. pylori* LPS recognition by TLRs. In AGS cells the induction of the TLR4/MD2 complex by *H. pylori* is dependent on the integrity of lipid rafts, where *H. pylori*, TLR4 and ceramide co-localize.[42] Furthermore, *H. pylori* LPS can induce the recruitment of TLR2 to lipid rafts and trigger the formation of receptor clusters involving TLR1, CD11b/CD18 and CD36 in human vascular endothelial cells.[43]

Several studies have demonstrated that *H. pylori* LPS only signals via TLR2[44-47] while others demonstrated signaling via TLR4.[32,48] Despite these controversial results it has been established that both TLR2 and 4 play a role in *H. pylori* LPS-mediated immune responses. Yokota et al.[49] were able to provide an experimental link between TLR2 and TLR4 signaling in a gastric epithelial cell line. They show that *H. pylori* LPS binding to TLR2 activates the MEK1/2-ERK1/2 pathway which leads to NF-Y-mediated transcription of TLR4 and results in IL-8 secretion.[49]

Overall, recognition of *H. pylori* antigens by epithelial cells culminates in chemokine secretion (IL-8, CXCL1, CXCL2, CXCL3 and CCL20) and subsequently leads to the recruitment of neutrophils, eosinophils, monocytes, dendritic cells and macrophages.[50,51] Macrophages located at sites of neutrophil infiltration in the lamina propria, gastric epithelium infiltrated by neutrophils as well as neutropils themselves have been reported to express high levels of Gro-α and IL-8, two neutrophil-attractant chemokines.[52] Furthermore, the upregulation of MIP-3α expression in gastric epithelial cells induces an influx of myeloid dendritic cells (DC) into the lamina propria as demonstrated in neonatally thymectomized BALB/c mice.[53]

In the following section we will discuss the current knowledge on sensing of *H. pylori* by innate immune cells and how this paves the way for the mixed pro- and anti-inflammatory adaptive immune responses typically observed in *H. pylori* infection.

Innate immune responses to *H. pylori*

In cultured human neutrophils *H. pylori* initiates an early innate immune response, which is partially mediated by TLR2 and TLR4, and characterized by rapid upregulation of IL-8, IL-1β and TNF-α followed by an increase in IL-10 production.[54] Furthermore, *H. pylori* NapA, an agonist of TLR2, induces IL-12 and IL-23 secretion by neutrophils as well as monocytes and elicits an antigen-specific Th1 response in the gastric mucosa of *H. pylori* infected patients.[55] Recognition of *H. pylori* PAMPs by antigen presenting cells, triggers the production of pro- and anti-inflammatory cytokines including IL-1, IL-6, IL-8, IL-10, IL-12 and type 1 IFN.[56] Rad et al.[57] identified 3 gene clusters that were upregulated in *H. pylori*-stimulated DC, i. MyD88 independent and MyD88-dependent mediated by ii. surface TLRs and iii. endosomal TLRs (Fig. 2). MyD88-independent expression of type-I IFNs and interferon-stimulated genes in DC is elicited by binding of *H. pylori* RNA to RIG-I (Fig. 2).[57] The main surface PRRs involved in *H. pylori* sensing by DC are TLR2 and to a lesser extent TLR4.[57] Both surface TLRs play a dual role in *H. pylori* pathogenesis. Although TLR2 signaling participates in the induction of pro-inflammatory responses, transcriptome analysis of *H. pylori*-treated DC also revealed a TLR2-dependent anti-inflammatory signature in mice.[57] Indeed, the lack of TLR2 in *H. pylori* infected mice induced a higher IFN-γ-mediated Th1 response and lower Treg/Th17 cell responses resulting in increased bacterial clearance at the expense of lesion development.[58] In human DC this dual pro-and anti-inflammatory role seems to be conferred by TLR4 which has been shown to be the main contributor to *H. pylori*-induced IL-10 and IL-12p70 mediated Th1 induction. Neutralization of TLR-4 led to reduced IFN-γ, IL-17A and IL-10 secretion as well as FOXP3 expression in *H. pylori*-primed DC-T cell co-cultures.[59] The endosomal TLR9 recognizing *H. pylori* DNA contributes significantly to the cytokine response (IL-6, IL-12) elicited by the bacterium. Interestingly, it also plays a role in the suppression of *H. pylori*-induced gastritis in the early phase by downregulating Th1 cytokines mediated by IFN-α (Type-I IFN).[60]

Another immune sensing mechanism playing an important role in immune responses toward *H. pylori* is the inflammasome. As a multi-protein complex it mediates the activation of CASP1 which promotes the secretion of the pro-inflammatory cytokines IL-1β and IL-18. Members of the NOD-like receptor family including NLRP1, NLRP3 and NLRC4 and the adaptor ASC are critical components of the inflammasome

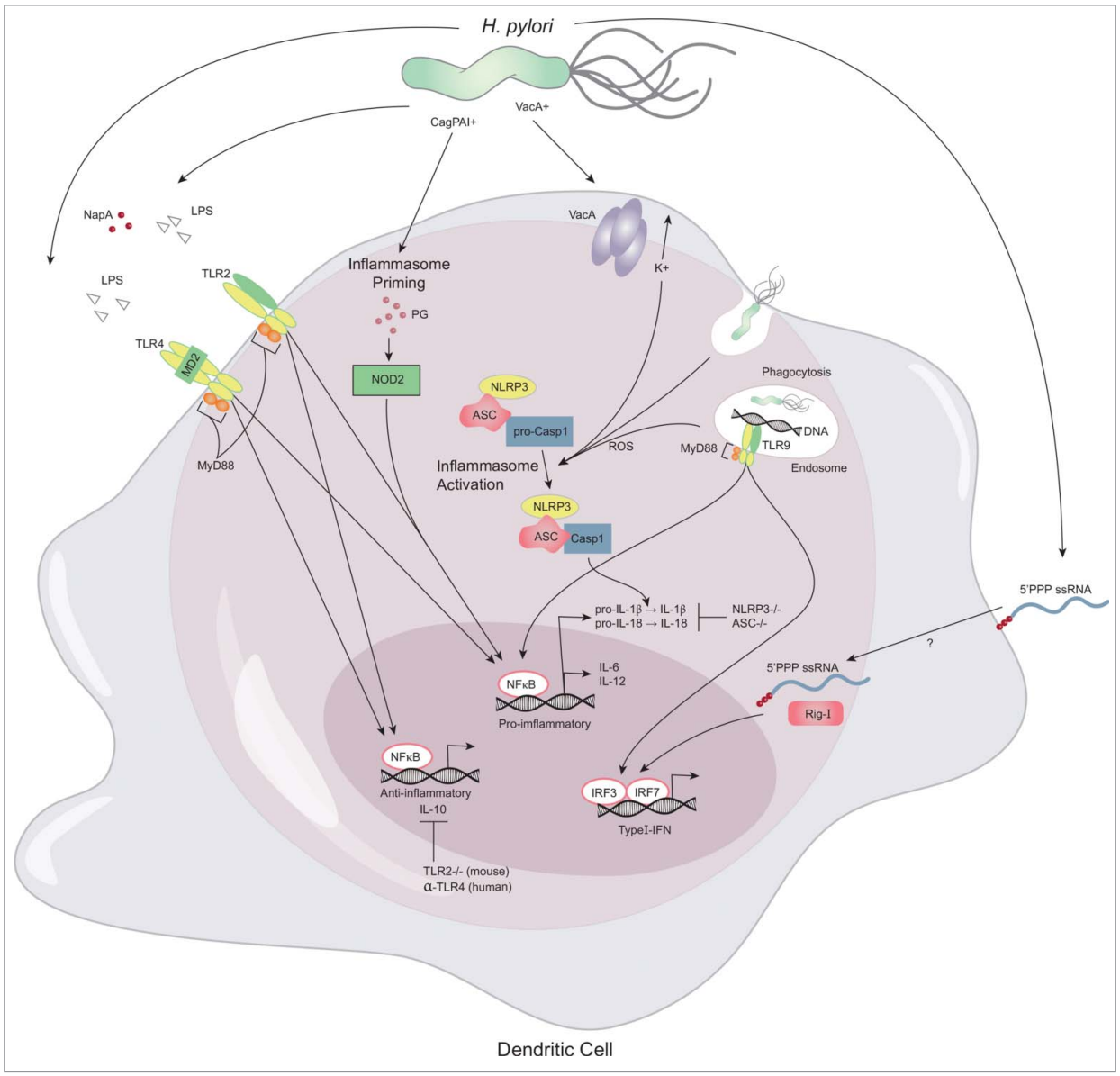

Figure 2. Innate immune sensing of *H. pylori*. The innate immune response to *H. pylori* is initiated by epithelial cells and tissue resident macrophages. Upon chemokine secretion dendritic cells are recruited to the gastric mucosa, where they encounter, recognize and process various known and unknown *H. pylori* antigens. Myd88-dependent TLR2 (LPS, NapA) and TLR4 (LPS) antigen binding results in both pro- and anti-inflammatory cytokine production which reflects the complexity of the immune response initiated by *H. pylori*. Following phagocytosis bacterial DNA is bound by TLR9 on endosomes and induces transcription of pro-inflammatory cytokines and type-I interferons (IFN) through NFkB and IRF7/IRF8, respectively. Bacterial RNA is recognized by RIG-I and elicits Myd88-independent signaling to induce transcription of type-I IFN. Furthermore, inflammasome priming is mediated by binding of peptidoglycan (PG) to NOD2 and subsequent activation of NFκB-mediated transcription of pro-IL-1β. Subsequently, the inflammasome complex NLRP3/ASC/pro-Casp1 is activated by potassium efflux and phagocytosis-induced ROS production, which results in Casp1-mediated cleavage of pro-IL-1β to IL-1β.

which link microbial and endogenous danger signals to caspase 1 (CASP1) activation.[61] In the context of *H. pylori* infection, the concomitant activation of TLR2 and NOD2 has been shown to induce pro-IL-1β expression and subsequent IL-1β secretion by *H. pylori*-infected DC. Dependency on CASP1 activation through priming of the NLRP3 inflammasome was demonstrated *in vitro* (Fig. 2).[62] A recent study by Semper et al.[63] further corroborates these findings and identifies potassium influx, phagocytosis and ROS production as mechanisms involved in *H. pylori*-mediated NLRP3 activation. Adhesion of *H. pylori* to the host cell and the *cag*PAI, but not CagA, were important for inflammasome activation in murine DC

and human monocytes/macrophages (Fig. 2). Moreover, *H. pylori* infected NLRP3-deficient mice showed increased bacterial colonization and decreased inflammation associated with decreased levels of IL-1β, IL-18, IL-17 and IFN-γ in the gastric mucosa.[63] Similarly ASC-deficient mice showed higher colonization and less inflammation upon *H. pylori* infection.[64]

Furthermore, the expression of NLRP12 and NLRX1, two negative regulators of NF-κB, were downregulated in THP-1-derived macrophages upon infection with the highly virulent *H. pylori* strain GC026 isolated from a gastric cancer patient. In concordance, NF-κB and several of its target genes including IFNB1, IL12B, IL6, TNF, CXCL1, CXCL2, CCL5, PTGS2 and BIRC3 were upregulated.[65] In line with these findings, we recently validated that intracellular *H. pylori* modulates the dynamics of NLRX1 and NLRC3 expression, another regulatory NLR, and simultaneously induces NF-κB responsive genes in bone marrow derived macrophages. Interestingly, NLRX1 was necessary for maintaining high levels of intracellular *H. pylori* at the cellular level and in a murine model of infection.[34] The contribution of NLRX1 in modulating host immunity to *H. pylori* may be associated with the combined effect of disruption of RIG-I signaling and inhibition of NF-κB activation by TRAF6 as observed in other infectious disease models, or a yet undiscovered pathway.[66,67] Suppression of regulatory NLRs is associated with worsened immunopathology in both immune mediated and infectious diseases but mechanisms of negative regulation have not been described.[68-70]

The following section reviews the molecular mechanisms and complex cellular interactions that underlie the pro-and anti-inflammatory adaptive immune responses to *H. pylori* and how this contributes to chronic gastritis.

Th1/Th17 responses to *H. pylori* infection

Consistent with the induction of Th1 and Th17 responses, *H. pylori*-treated DC stimulate the secretion of IFN-γ and high levels of IL-17 by CD4+ T cells. Furthermore, murine splenic macrophages stimulated with *H. pylori* signficantly upregulated gene expression of Th1- and Th17-inducing cytokines including IL-6, TGF-β, IL-23 p19, IL-12/IL-23 p40 and IL-12 p35 (Fig. 3).[71] Even though *H. pylori*-pulsed DC secrete only low levels of IL-12 (p35/p40), robust IL-23 (p19/p40) expression is induced.[72] Importantly, IL-23 was also detected in gastric myeloid DC of *H. pylori*-infected patients[72] and expression of IL-23 p19 was increased in gastric epithelial cells of patients with *H. pylori*-associated gastritis, suggesting a potential role for epithelial cells in shaping mucosal immunity.[73] IL-23 belongs to the IL-12 family of cytokines and is known to promote the expansion and survival of Th17 cells.[74] Indeed, blocking of endogenous IL-23 in cultures of lamina propria mononuclear cells from *H. pylori*-infected patients inhibited STAT3 phosphorylation and consequently diminished IL-17 secretion.[75] The virulence factors CagA and CagE have been implicated in the induction of IL-17 and IL-23 secretion further corroborating the significance of the *cag*PAI in *H. pylori* immunopathology. The cytokine BAFF, released by macrophages upon *H. pylori* infection, has recently been implicated in the induction of *H. pylori*-mediated Th17 responses in humans. BAFF has been shown to induce Th17 differentiation directly or indirectly through the creation of a pro-Th17 cytokine milieu by innate immune cells.[76]

Regulatory T cell responses to *H. pylori*

Despite the induction of mixed pro-inflammatory T cell responses, the immune system is unable to clear *H. pylori* resulting in life-long colonization. An underlying regulatory immune response which is induced by known and unknown bacterial factors and executed by regulatory T cells has been attributed to *H. pylori*'s ability to persist in its host. Although *H. pylori*-pulsed DC increase the production of IL-12 and induce secretion of Th1 cytokines (TNF-α, IFN-γ) as well as proliferation of murine splenocytes (Fig. 3), this response is much lower compared to DC pulsed with the Gram-negative bacillus *Acinetobacter iwoffii*. A heat stable soluble factor released by *H. pylori* has been found to suppress IL-12 secretion by *A. iwoffii*-stimulated DC.[77] Another study recently demonstrated that *H. pylori* VacA suppressed IFN-β and IL-12 expression in murine bone marrow derived macrophages stimulated with *Lactobacillus acidophilus*.[78] Furthermore, gastric and intestinal stroma-conditioned medium has been shown to down-regulate DC responsiveness to *H. pylori in vitro*. A yet unknown stromal factor inhibited monocyte-derived DC activation and impaired IL-12 release upon *H. pylori* infection consequently resulting in the reduced ability of

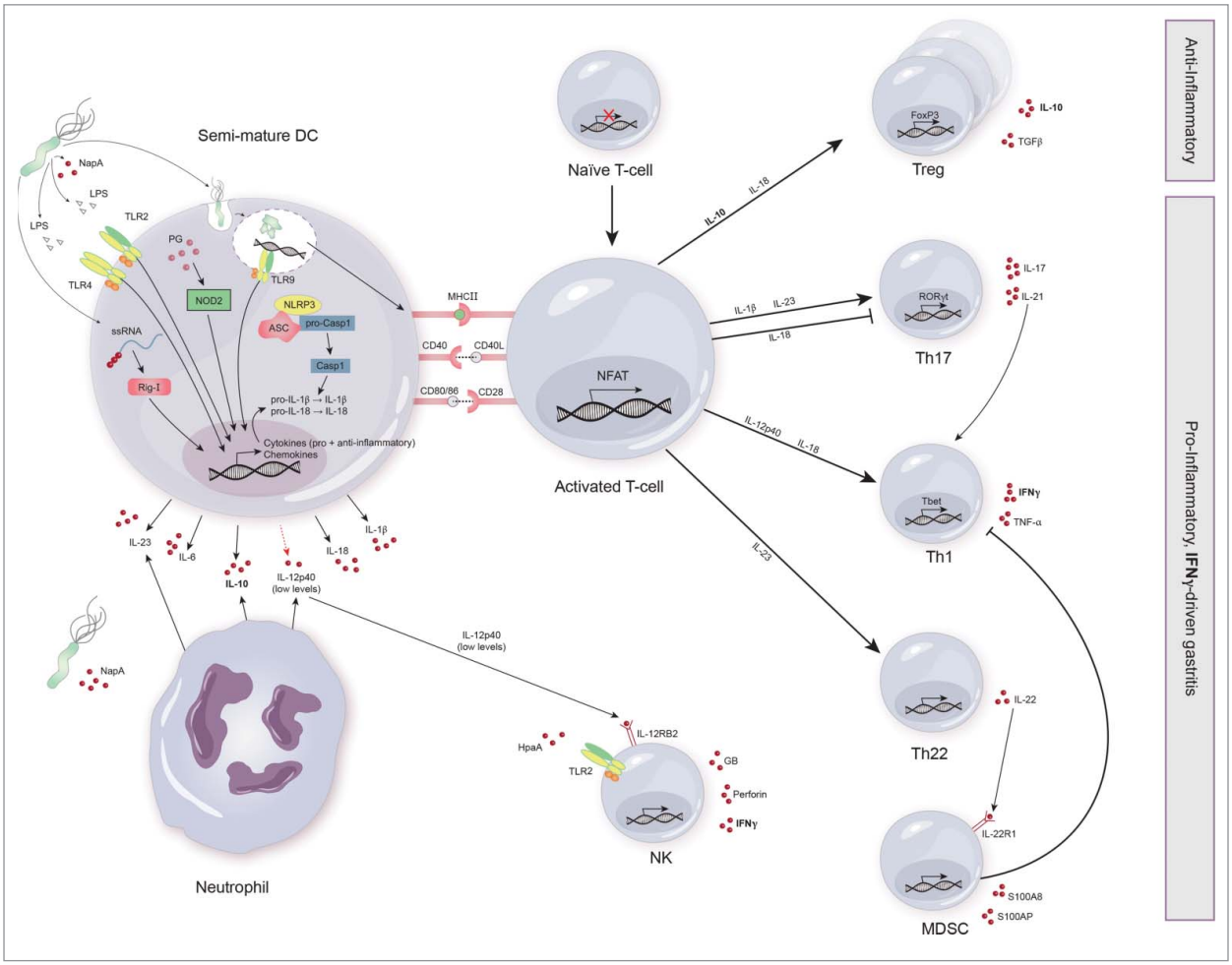

Figure 3. Shaping adaptive immune responses to *H. pylori*. Dendritic cells (DC) encountering *H. pylori* have been shown to possess a semi-mature phenotype. The adaptive immune response to *H. pylori* is characterized by a mixed anti- and pro-inflammatory response. Upon pathogen receptor recognition, DC and neutrophils secrete a number of cytokines including IL-1β, IL-6, IL-10, IL-18, IL-23 and low levels of IL-12p40. These cytokines and concomitant presentation of *H. pylori* antigens by semi-mature DC to naive T cells via MHC-II results in the expansion of *H. pylori*-specific Treg. Furthermore, T cells differentiate into Th17, Th1 and Th22. The secretion of IL-22 by Th22 induces the secretion of anti-bacterial proteins by MDSC (monocytic-myeloid derived suppressor cells). IL-21 secretion by Th17 supports Th1 differentiation and thus maintains the Th17/Th1 axis during gastritis. In contrast, MDSC exert an inhibitory effect on Th1 cells. Upon binding of HpaA to TLR2 and IL-12RB2 signaling, NK cells secrete granzyme B (GB), perforin and IFN-γ. Importantly, IFN-γ secreted by NK cells, Th1 cells and possibly other cell types (CD8+ T cells) has been identified as the main driver of gastritis.

H. pylori-pulsed DC to induce an IFN-γ mediated Th1 response.[79] Together, these studies suggest that both *H. pylori*- and host-derived factors might hinder the induction of a robust Th1 response through the suppression of IL-12 release by DC.

In line with the observation that *H. pylori* interferes with DC maturation *in vitro*, DC infiltrating the gastric mucosa of human *H. pylori* carriers exhibit a semimature DC-SIGN^{+}HLA-DR^{hi}CD80^{lo}CD86^{lo} phenotype.[80] Specifically, *H. pylori* can trigger a reprogramming of DC by downregulating MHC-II, inducing IL-10 and inhibiting IL-12 secretion thereby inducing *H. pylori* specific Treg cells.[81-83] CagA has been identified as the main virulence factor responsible for induction of semi-mature DC, IL-10-mediated STAT3 activation and subsequent Treg induction.[84] Increased numbers of Treg cells are typically found in the stomachs of infected patients, suggesting that they play an important role in regulating inflammatory responses to *H. pylori*.[85] In children, *H. pylori* infection favors the induction of mucosal Treg responses over Th17 and is thus associated with reduced gastric inflammatory lesions compared to adults.[86,87] Overall, patients with fewer or less functional Treg cells are more likely to develop peptic ulcers and are afflicted by more intense gastritis.[88] B cells also play a regulatory role by

promoting IL-10 production in co-cultured CD4+ cells and subsequent conversion into a T regulatory 1 (Tr1)-like phenotype.[89] The importance of Treg cells for the control of *H. pylori*-induced gastritis has been highlighted in several studies. Adoptive transfer of C57BL/6 derived CD4+ splenocytes into *H. pylori*-infected RAG-KO mice lacking Treg induced more severe gastritis compared to *H. pylori*-infected C57BL/6 mice.[90] Moreover, depletion of Treg cells resulted in decreased bacterial colonization at the expense of increased gastric inflammation.[91]

One host cell factor on the intersection between pro- and anti-inflammatory responses toward *H. pylori* is the enzyme CASP1. Upon activation by the inflammasome complex it dynamically regulates immune responses through IL-1β and IL-18, respectively.[92] IL-1 signaling is required for the induction of Th17 responses[72] and IL-18 together with IL-12 is known to induce Th1 responses (reviewed in [93]). Albeit its pro-inflammatory role, the presence of IL-18 prevented excessive immunopathology[92] and induced *H. pylori*-specific tolerance through the induction of Treg differentiation (Fig. 3), while its absence promoted unrestricted Th17 responses to *H. pylori* in mice.[80] Thus, *H. pylori*-induced IL-18 secretion exerts an immune-regulatory function by counteracting the activities of IL-1β.

Drivers of *H. pylori*-induced gastritis

To better understand, prevent and treat *H. pylori*-mediated disease one should identify the main factors involved in the development and sustainance of gastritis. A recent study by Gray et al.[90] has shed further light on the complex interactions leading to *H. pylori*-induced gastritis. They determined that *H. pylori*-specific CD4+ T cells, innate immune cells expressing the common cytokine gamma chain and IFN-γ were essential for the development of gastritis in mice, whereas Th1 cells were not. This study suggests that IFN-γ secreted by other cell subsets (possibly functional innate immune cells) is the main driver of gastritis (Fig. 3).[90] Thus far, NK cells and CD8+ cytotoxic T cells have been identified as additional source for IFN-γ in *H. pylori* infection. Yun et al.[94] demonstrated that stimulation of human NK cells with *H. pylori* antigens induced IFN-γ production and increased the expression of granzyme B and perforin. Interestingly, *H. pylori*-induced IFN-γ secretion was dramatically increased in the presence of small doses of IL-12 p40. This synergistic effect was dependent on antigen exposure prior to addition of IL-12 and was associated with increased expression of IL-12 receptor β 2 (Fig. 3).[94] *H. pylori* specific membrane bound lipoprotein A (HpaA) has been implicated in the induction of IFN-γ secretion by NK cells, which was dependent on the recognition by TLR-2 and MyD88 as well as p38 MAPK signaling (Fig. 3).[95] CD8+ T cells have also been implicated as a source for IFN-γ. A study assessing proliferation and cytokine secretion of circulating T cells from *H. pylori*-infected patients demonstrated induction of IFN-γ secretion from both CD4+ T cells and CD8+ T cells upon *H. pylori* antigen exposure. Interestingly, CD8+ T cells secreted even more IFN-γ than CD4+ T cells on a per cell basis.[96] Studies in *H. pylori* infected children with grade I-III gastritis showed an increased number of CD8+ T cells in the gastric epithelium and lamina propria.[97,98] Another study demonstrated an increase of CD8+HLA-DR+ chronically activated memory T cells in peripheral blood of *H. pylori*-colonized children with duodenal ulcers.[99] We have recently demonstrated the expansion of circulating NK cells, CD8+ T cells and concomitant increases in cytotoxic markers including CD16, granzyme B and perforin in a pig model of *H. pylori* infection.[100] Thus, a cytotoxic cell response might play a major role in *H. pylori*-mediated pathology at least in part due to the cytokine response induced by the bacterium.

While it has been established that IL-17A secretion and Th17 cells play a role in *H. pylori*-mediated gastritis[71] their contribution is less dramatic than that of IFN-γ.[90] Interestingly, recent studies have established an association between Th17 and Th1 responses. IL-17 deficiency in mice signficantly reduced *IFN-γ*, *T-bet* and *IL-12 p40* mRNA expression in gastric tissue of *H. pylori* infected animals suggesting that the Th17/IL-17 pathway plays a role in driving Th1 responses.[71] Further to the association between Th17 and Th1 cells, Caruso et al.[75] demonstrated that IL-23 does not only enhance IL-17 but also IFN-γ secretion by *ex vivo* stimulated normal human gastric lamina propria mononuclear cells. Blocking IL-23 in these cells significantly reduced IL-17 and IFN-γ secretion.[75] Another molecule that has been implicated in *H. pylori*-induced gastritis is IL-21. IL-21 is a functional hallmark of T follicular helper (Tfh) cells, can also be produced by NKT cells, Th17 cells, and has been linked

to Th1 and Th17 differentiation at the expense of Treg cell formation.[101,102] Of note, we have recently identified the accumulation of cells with a Tfh phenotype (CD4+ T cells expressing CXCR5, ICOS, PD-1, BCL6, and IL-21) in the mesenteric lymph nodes and gastric lamina propria upon *H. pylori* infection in C57BL/6 mice.[103]

Deficiency of IL-21 in *H. pylori*-infected mice resulted in increased bacterial burden and decreased inflammation characterized by highly reduced infiltration of neutrophils, B cells, CD4+ and CD8+ T cells into the stomach. The study also presented data showing that the mRNA expression of chemokines and cytokines including TNF-α, IL-1β, IL-17A and IFN-γ was inhibited in IL-21-deficient mice compared to their wild-type littermates upon *H. pylori* infection. This was associated with reduced expression of TBX1 and RORγt as well as reduced levels of activated STAT1 and STAT3. Thus, IL-21 contributes to the maintenance of both Th1 and Th17 responses.[104]

Another recently described T cell subset that contributes to *H. pylori* induced gastritis are Th22 cells. Zhuang et al.[105] show that Th22 cells are enriched in the gastric mucosa of *H. pylori*-infected patients and mice. They demonstrated that IL-23-secreting DC encountering *H. pylori* CagA+ strains highly induce secretion of IL-22 by CD4+ T cells. This leads to the recruitment of CXCR2+ monocytic-myeloid derived suppressor cells (MDSC) into the gastric mucosa via IL-22R1 signaling and subsequent secretion of CXCL2 by gastric epithelial cells. Furthermore, MDSC secrete the two pro-inflammatory proteins S100A8/S100A9 upon IL-22 stimulation and suppress Th1 cell development. (Fig. 3).[105]

Mechanisms of immune evasion

Consistent with its ability to chronically colonize the gastric microenvironment, *H. pylori* is equipped with efficient ways to evade recognition and clearance by the immune system. This is in part attributed to the expression of modified, less immunogenic PAMPs and to the secretion of certain pathogenicity factors, which alter the function of innate immune cells. Hence, *H. pylori* has the ability to evade, attenuate and modify innate and adaptive immune responses.

Modification of LPS, e.g., by adding positively charged substituents or removing phosphate groups from the backbone, renders *H. pylori* much less immunogenic than LPS from other gram-negative enteric bacteria such as *Escherichia coli*.[106] The LPS component lipid A has been shown to bind to and activate the hTLR4-MD2 complex. Lipid A dephosphorylation by the enzymes LpxE and LpxF results in attenuated hTLR4-MD2 activation and confers resistance to the actions of cationic antimicrobial peptides[48] thereby limiting the host's immune response and favoring *H. pylori*'s persistence.

Another mechanism of immune evasion is *H. pylori*'s ablity to manipulate the function of innate immune cells. After successful phagocytosis by neutrophils, *H. pylori* disrupts the NADPH oxidative system thus enabling unopsonized *H. pylori* to escape phagocytic killing.[107] Furthermore, *H. pylori* impairs neutrophil migration by decreasing the expression of CXCR1 and CXCR2.[108] *H. pylori* induces the expression of arginase 2 (Arg2) in lamina propria macrophages thereby restricting iNOS upregulation, NO production and enhancing macrophage apoptosis. Accordingly, the expression of IFN-γ, IL17A and IL-12 p40 was increased and levels of IL-10 were decreased in the gastric antrum of Arg2-/- mice.[109] Cyclooxygenase (COX)-2 is a key enzyme in arachidonic acid metabolism and induced by *H. pylori* via MEK/ERK signaling independent of the T4SS.[110] Inhibition of COX-2 and its metabolic product prostaglandine E2 (PGE2) resulted in the increased production of IL-12 and IFN-γ and a decrease in IL-10 levels upon *H. pylori* infection[111] suggesting that it contributes to the anti-inflammatory response against the bacterium.

Survival of *H. pylori* inside lamina propria macrophages[112] also contributes to its ability to evade elimination by manipulating phagosome and autophagosome maturation.[113,114] It impairs the antimicrobial activity of macrophages through the production of catalase[115] and inhibits NO production by decreasing the availability of L-arginine through the bacterial arginase *rocF*[116,117] as well as inhibiting L-arginine uptake by inducing spermine oxidase.[118] Once engulfed, *H. pylori* is present in phagosomes[119] and escape from phagocytic killing is dependent on *cag*PAI.[120,121] VacA can arrest phagosome maturation by preventing its fusion with lysosomes, thus contributing to the pathogen's persistence inside the cell.[121] *H. pylori* uses host cholesterol to synthesize cholesterol-α-D-glucopyranoside (CG). This process is mediated through the enzyme cholesterol-

α-glucosyltransferase (CGT), encoded by *capJ*. The presence of *capJ* has been shown to confer resistance to phagocytic endocytosis, phagolysosome-fusion and -maturation. Furthermore, the synthesis of CG by CGT is responsible for retarded entry through the lipid-raft endocytic route and prolonged intracellular survival in a PI3K-dependent manner.[122] *H. pylori* induces the formation of autophagosomes which are adapted for its replication and thus contribute to increased antimicrobial resistance.[113] In primary human macrophages, virulent *H. pylori* strains can survive for an extended period due to the formation of megasomes, which are large structures arising from homotypic fusion of phagosomes.[123]

Additionally, *H. pylori* can directly limit adaptive immune responses by limiting nutrients essential for T-lymphocytes. The depletion of L-arginine by *H. pylori* arginase decreases the expression of CD3 zeta chains associated with the T cell receptor, thus inhibiting T cell proliferation.[124] *H. pylori* γ-glutamyltranspeptidase depletes extracellular glutamine and consequently inhibits T lymphocyte proliferation, impairs activation and inhibits cytokine secretion involving IRF4.[125] Endocytosis of VacA into activated human primary T cells[126] inhibits cell proliferation and the clonal expansion of *H. pylori* antigen specific T-cells.[127] It has also been shown that VacA can block T cell activation by preventing influx of extracellular calcium and subsequent nuclear translocation of NF-AT and by stimulating disordered actin polymerization through Rac activation.[128]

The beneficial role of *H. pylori* in extra-gastric disease

Since the identification of *H. pylori* as a causative agent of peptic ulcers and gastritis in 1983[4], the bacterium has been considered primarily a pathogen. However, infection with *H. pylori* is asymptomatic in 85% of individuals, only 15% develop symptomatic peptic ulcer, and less than 1% develop gastric cancer.[129] A potential role of *H. pylori* as a gastric commensal or symbiotic bacterium is beginning to emerge in recent years. It has been demonstrated that *H. pylori* colonization even exerts beneficial effects in allergic diseases, such as asthma[130] and eczema,[131] as well as gastroesophageal reflux disease,[132] obesity and diabetes.[133] *H. pylori*'s ability to evade the immune system and to persist in the gastric mucosa through induction of Treg cells, regulatory CX3CR1+ mononuclear phagocytes, and anti-inflammatory molecules as discussed above lends further support to its potential role as a commensal organism.

It has been demonstrated that CD4+CD25+ T cells from *H. pylori*-infected neonatal WT mice are able to prevent the development of allergen-induced asthma in mice.[80] Furthermore, *H. pylori* NapA has been shown to skew the immune response from a Th2 to a Th1 response in a mouse model of OVA-induced allergic asthma.[134] In the context of immune-mediated diseases *H. pylori* infection protects against *Salmonella typhi*-induced colitis by suppressing Th17 responses in the lower intestinal tract and upregulating IL-10 expression in lymph nodes.[135] Subsequent studies, demonstrated how *H. pylori* DNA decreases pro-inflammatory cytokine production by DC and attenuates sodium sulfate (DSS)-induced colitis in mice.[136] Using a mouse model of DSS-induced and T-cell-transfer-induced colitis Engler et al.[137] recently demonstrated a beneficial effect of *H. pylori* infection on clinical and histopathological features of colitis. They showed that *H. pylori*-mediated protection against DSS-induced colitis was dependent on the NLRP3 inflammasome and IL-18 signaling.[137]

Results from a study on the protective role of *H. pylori* in obesity, provide *in vivo* evidence that gastric colonization with a *cag*PAI negative *H. pylori* strain ameliorates glucose tolerance possibly by activating the transcription factor PPAR-γ. The presence of *cag*PAI negative *H. pylori* modulated appetite-controlling hormones (i.e., ghrelin and leptin), suppressed adipose tissue inflammation, and ameliorated glucose homeostasis. Interestingly, these effects were abrogated upon conditional knockout of PPAR-γ in immune- and epithelial cells.[133] The inverse relationship between obesity and *H. pylori* colonization was recently studied in a North American cohort of inner city children revealing a 50% reduction in the odds of being obese when colonized with *H. pylori*.[138]

Recent epidemiological and clinical data indicate that the incidence of obesity[139] and inflammatory bowel disease (IBD)[140] is greater in areas with low prevalence of *H. pylori* infection. Whether this is due to a „spontaneous eradication" of *H. pylori* caused by immunomodulatory drugs and antibiotics taken by IBD patients or due to an actual protective effect of *H. pylori* is still controversial.[141] A study supporting

the causal association of *H. pylori* and IBD, reports that the rising incidence of IBD in *H. pylori*-endemic regions corresponds to the use of anti-*H. pylori* therapy for the treatment of peptic ulcer disease.[142] Therefore, the evidence for *H. pylori*'s beneficial effects in allergic, immune-mediated and metabolic diseases seriously questions the validity of widespread *H. pylori* eradication strategies. Thus far, little is known about the relevance of virulence factors in *H. pylori*-mediated protection and this is likely to differ between diseases. In a study using data from 7663 participants of a large US National Health and Nutrition Examination Survey (NHANES III) Chen and Blaser[12] show that the infection with a more virulent CagA+ *H. pylori* strain was inversely associated with ever having had asthma in younger study participants. The beneficial effect of CagA+ strains in allergic disease and asthma can be at least partly explained by the increased IL-10 responses inducing semi-mature DC and higher Treg responses.[84,143] To date, no conclusive association between CagA status and Crohn's disease has been made. Although a high frequency of Crohn's disease patients was infected with CagA+ strains, this was similar to controls.[144] Thus, further pre-clinical and clinical studies as well as modeling studies are needed to strengthen and mechanistically characterize *H. pylori*'s role as beneficial member of the gut microbiota.

H. pylori infection provides an excellent avenue to probe mucosal immune responses and study mechanisms of immunoregulation and host tolerance that reduce the negative impact of infection on host fitness. Unlike resistance mechanisms, tolerance does not directly affect microbial burden. Rather, tolerance decreases the host susceptibility to tissue damage, or other fitness costs, caused by the pathogens or by the immune response against them. Given the co-evolution of *H. pylori* and its human host, studying mechanisms of *H. pylori*-host interaction may yield novel insights on how the human host can protect itself from infectious diseases by reducing the negative impact of infection on host fitness, independently of modulating the microbial burden. New integrative analyses can help synthesize and transform data, procedural knowledge and theory into information processing representations of immune responses to *H. pylori* with the potential to yield transformative systems-wide immunological knowledge.

Computational modeling to advance our understanding of the complex interactions between *H. pylori* and the gastric immune system

The interaction between *H. pylori* and the stomach mucosa is a complex and dynamic process. Computational modeling can be used to support pre-clinical and clinical research in *H. pylori* and to identify emerging behaviors of the immune response. We developed a tissue-model of the immune response to infection that represents the gastric lumen, epithelium, lamina propria and lymph nodes.[145] The model was calibrated with data from an *in vivo* time-course study of *H. pylori*-challenged mice and reproduced the effector and regulatory pathways in the gastric lamina propria, including the induction of a Th17 response, a dominant Th1 response and high levels of mucosal Treg.[145] Computational models can be used to study new therapeutic targets in the context of disease processes. To shed light on the potential role of the transcription fator PPAR-γ in modulating host reponses to *H. pylori* we used a loss-of-function approach. *In silico* results predicted a predominance of Th17 and Th1 responses in cell-specific PPAR-γ knockout compared to WT mice.[145] Sensitivity analysis was used to rank immunological parameters by their relevance in the system's dynamics and outcomes. Global sensitivity analysis has revealed that epithelial cells and macrophages have the highest influence in the mucosal immune response to *H. pylori*. The prediction of the impact of epithelial cells support the model itself, since most of *H. pylori* is found in contact with epithelium,[24,146,147] and the epithelial cell has been implicated in the initiation of innate responses (as discussed above).

With regards to macrophages, the analysis predicts the initial presence of macropage precursors, followed by the accumulation of a subset of M2 regulatory cells, which peaks between days 21 and 35. Interestingly, through the guidance of our computer modeling work, we have recently identified a CX3CR1+CD64+CD11b+F4/80+ subset of macrophages[148] (unpublished data) that starts accumulating in the stomach of mice at day 15, peaks around day 24 and it is maintained at a steady state for at least 16 weeks. These macrophages produce IL-10 and by depletion studies we have demonstrated that they facilitate colonization by *H. pylori*.

In addition to the tissue-level computational model that can scale up to 10 billion cells and molecules at a time, we have engineered a model representing signaling events leading to CD4+ T cell differentiation and plasticity.[104,149,150] Our cell-based model is generic in the sense that it represents CD4+ T cell differentiation out of any particular context,[149,150] although it can be recalibrated with data obtained during infection. To investigate the role of IL-21 during *H. pylori* infection, we re-calibrated the model with CD4 data obtained from mice infected with *H. pylori* and used to construct an IL-21 knockout system.[104] The main prediction of the model was the reciprocal interplay between IL-21 and IL-10 whereby, in the context of an *H. pylori* infection, IL-21 promotes differentiation of CD4+ T cells into Th1 and Th17 and opposes IL-10, and vice versa: lack of IL-21 turns the system in favor of IL-10. The predictions of the model were the validated *in vivo* using IL-21 KO mice. Others have also used mathematical modeling to evaluate the expression of pro and anti-inflammatory cytokines, and how their levels are affected by sonic hedgehog (SHH),[151] a gene product that is upregulated in gastric parietal cells early post-*H. pylori* infection and has been proposed to be a macrophage chemoattractant.[152]

In summary, integrating computational and mathematical approaches to guide pre-clinical and clinical experimentation into the analysis of the host response to *H. pylori* can help accelerate the discovery of the mechanisms of action that explain the role of this microbe as pathogen or commensal microbe. Modeling and informatics tools that create information processing representations of immunological processes allow connecting immunology and microbiology to the world above the skin, testing interventions in virtual laboratories to guide human studies.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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