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Current Opinion in Immunology

Review

Innate immune activation and modulatory factors of *Helicobacter pylori* towards phagocytic and nonphagocytic cells

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Helicobacter pylori is an intriguing obligate host-associated human pathogen with a specific host interaction biology, which has been shaped by thousands of years of host-pathogen coevolution. Molecular mechanisms of interaction of *H. pylori* with the local immune cells in the human system are less well defined than epithelial cell interactions, although various myeloid cells, including neutrophils and other phagocytes, are locally present or attracted to the sites of infection and interact with *H. pylori*. We have recently addressed the question of novel bacterial innate immune stimuli, including bacterial cell envelope metabolites, that can activate and modulate cell responses via the *H. pylori* Cag type IV secretion system. This review article gives an overview of what is currently known about the interaction modes and mechanisms of *H. pylori* with diverse human cell types, with a focus on bacterial metabolites and cells of the myeloid lineage including phagocytic and antigen-presenting cells.

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Abbreviations: ADP, Adenosine-Diphosphate; ALPK1, Alpha Kinase 1; BM, Bone Marrow; BMP, Bone Morphogenetic Protein (growth factor); CagT4SS, Cag Type 4 Secretion System; cGAS, Cyclic GMP-AMP Synthase (signals together with STING); CIITA, Class II Major Histocompatibility Complex Transactivator; DC, Dendritic Cell; DC-SIGN, Dendritic Cell-Specific ICAM-3-Grabbing Non-Integrin 1 (alias: CLEC4L); ieDAP, D-gamma-Glu-m-Di-Amino Pimelic Acid (peptidoglycan metabolite, MAMP, NOD1 agonist); MALT, Mucosa-Associated Lymphoid Tissue; MAMP, Microbe-Associated Molecular Pattern (innate immune activator); MINCLE, Macrophage-Inducible C-Type Lectin (alias: CLEC4E); NLRP3, NLR Family Pyrin Domain Containing 3 (NOD-like receptor family member); NADPH, Nicotinamide Adenine Dinucleotide Phosphate; NFAT, Nuclear Factor Of Activated T cells; NF-κB, Nuclear Factor kappa B (transcription factor); NKT, Natural Killer T-cells; NOD, Nucleotide Oligomerization Domain (pattern recognition receptor class); PRR, Pattern Recognition Receptor (innate immune response); ROS, Reactive Oxygen Species; RIG-I, Retinoic Acid Inducible Gene I (intracellular RNA sensor); STING, Stimulator Of Interferon Response CGAMP Interactor 1 (nucleotide PRR); Thp-1, Cell line name (human monocytic cell line); TIFA, TRAF Interacting Protein With Forkhead Associated Domain; TLR, Toll-Like Receptor (pattern recognition receptor class)

Current Opinion in Immunology 2023, **82**:102301

This review comes from a themed issue on **Chronic Infections**

Edited by **Thomas Mertens**

For complete overview of the section, please refer to the article collection, "Chronic Infections"

Available online 16 March 2023

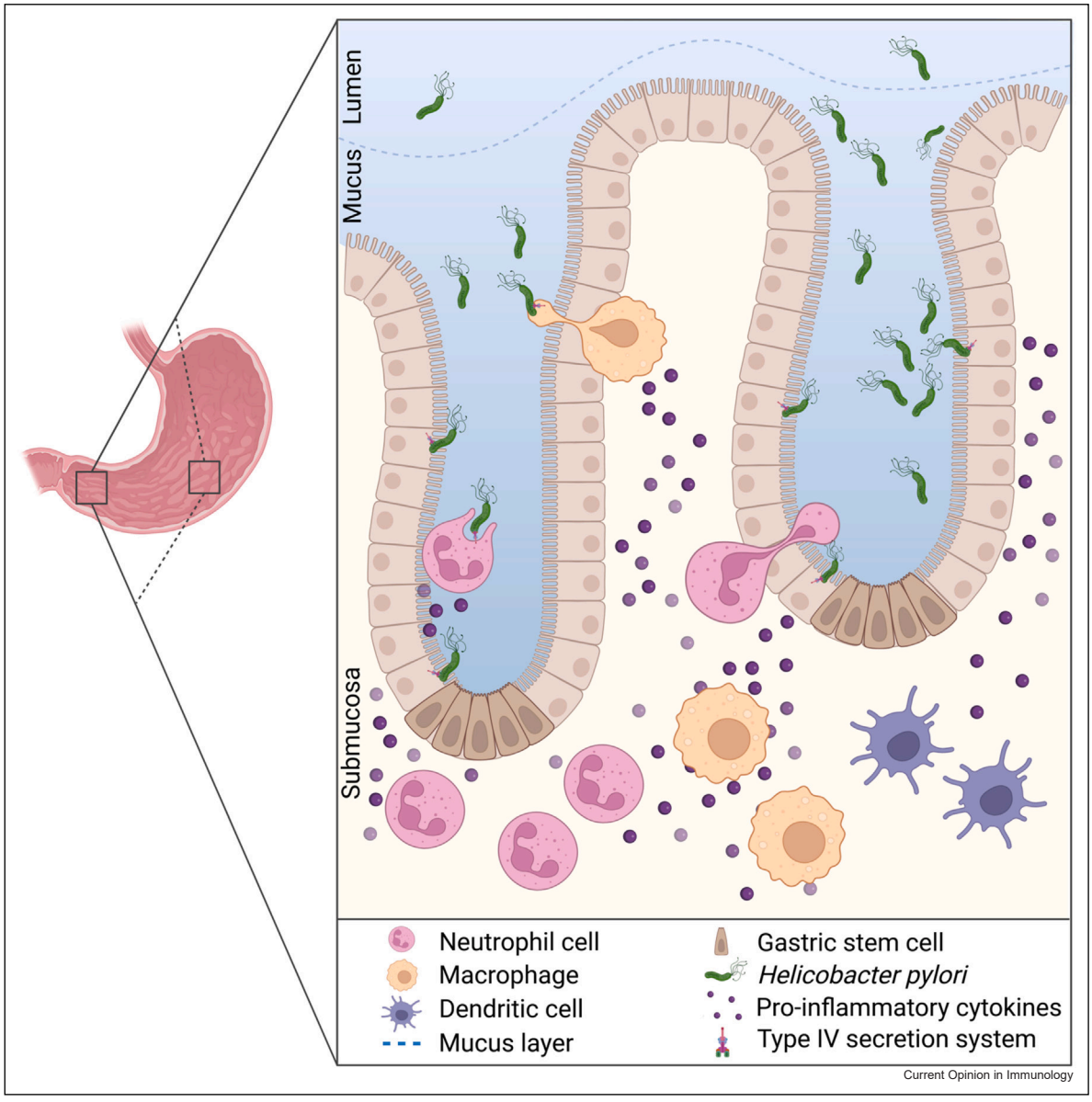
https://doi.org/10.1016/j.coi.2023.102301

Helicobacter pylori, its native niche in the stomach and its potential to contact various cell types of the epithelial, myeloid and lymphoid lineages

It is well established that the bacterium *Helicobacter pylori* is a chronic stomach pathogen of major global importance, since more than half of the world population carries this bacterial species in their stomachs [1]. Infected individuals usually show asymptomatic chronic gastritis, but some develop gastric ulcers and gastric cancer [2]. Disease progression seems, by association studies, to be determined by several key factors, among them host genetic predisposition, bacterial genotype, and environmental parameters [3]. Its colonization niche deep in the gastric mucus layer and within the gastric crypts [4,5] is characterized by the presence of specific cell types in addition to epithelial cells, including cells of the myeloid and lymphatic lineages [6–9] and a stem cell zone [10–12] (Figure 1).

During the *H. pylori* infection in its acute and chronic phases, gastric epithelial cells provide a first line of defense against the bacteria. One essential function of this bordering cell layer is to maintain a protective barrier that separates the acidic lumen and the potentially *H. pylori*-inhabited mucus layer from the underlying

Figure 1



A schematic overview of the stomach habitat of *H. pylori* is shown. *H. pylori* persists in the lower layers of the stomach mucus and in the gastric crypts, where it proliferates and forms microcolonies. In particular in the gastric crypts, *H. pylori* can get into contact with myeloid cell types with phagocytic and antigen-presenting properties and stem cells, as indicated. Its type IV secretion system promotes bacterial interaction with epithelial and nonepithelial cells and secretes various proteins and metabolites which can influence cell responses. Innate and adaptive lymphocytes are not shown in this scheme, since they are not in the focus of this article. The artwork of this figure was created by BioRender.

submucosal tissue compartment [8]. Besides their barrier function, gastric epithelial cells also alert and recruit phagocytic cells, for instance, neutrophils, dendritic cells, monocytes, and macrophages, to the sites of infection [6,13], which are probing the mucosa-adherent bacteria. Neutrophils are predominantly found attracted to submucosal gastric tissues close to infection sites, once *H. pylori* has activated proinflammatory signaling, which leads to the secretion of chemokines such as IL-8 [14,15] and Gro-alpha. In addition, gastric MALT develops during chronic *H. pylori* infection, including the organization of follicle-like structures with incoming B-cells and T-cells [16]. *H. pylori's* direct interaction with stem cells in the depth of the gastric crypts [4,17] has been proposed to facilitate carcinogenesis during chronic infection [18,19], and with possible involvement of gastric cancer stem cells [10,20,21].

Interaction of *H. pylori* with gastric epithelial cells and concomitant innate immune activation and evasion

The molecular interaction of *H. pylori* with epithelial cells, primarily of gastric origin, has been very well studied in the past 20 years, also thanks to well-established human gastric cell lines. *H. pylori* activates gastric epithelial cells by different innate immune pathways upon intimate cell adherence [22–25]. Adherence of *H. pylori* is by outer membrane protein autotransporter adhesins. The best-characterized among them are SabA and BabA, which interact with cellular glycans, sialyl-Lewis^{X}, and Lewis^{b}, respectively [26,27]. *H. pylori* adheres to gastric epithelial cells very specifically, using AlpA/B, SabA, and BabA/B [26,28–30]. Bacterial and/or host population-specific effects have been reported [31,32]. Not only surface glycans are engaged, in a partially human-specific manner, but also cell surface proteins, such as ceacams and integrins, and are also involved in the activity of the important *H. pylori* virulence module, the Cag type IV secretion system (Cag T4SS) [33–35]. Other cell surface receptors that may be bound by *H. pylori* [36,37] are still under investigation.

Primary bacterial adherence is required for cell-autonomous gastric epithelial cell immune responses [34,38], which is mediated by pattern recognition receptors (PRR) on the host cell side. Innate PRR that are addressed by *H. pylori* include, among others, TLR2 [39], TLR9 [40], NOD1 [23,41] and NLRP3 [42,43], however do not contribute strongly to bacterial attachment. In gastric epithelial cells, the activation of downstream proinflammatory or tolerogenic signaling pathways and their molecular mechanisms have been defined [25,44–46]. Interestingly, *H. pylori* largely evades TLR4 and TLR5 pattern receptor recognition, since the respective canonical bacterial surface ligands or microbe-associated molecular patterns (MAMPs) of *H. pylori*, lipopolysaccharide (LPS/lipid A for TLR4) and flagellins for TLR5, have evolved to low intrinsic activation potential [39,47–50]. The *cag* pathogenicity island (*cag*PAI) is the most-studied cell interaction and modulation module of *H. pylori* with respect to gastric epithelial cells. This large genetic element is located on the *H. pylori* genome in about 70% of all global isolates [33] and encodes for a complex membrane-spanning secretion system of the Type IV (T4SS) [51–54]. The presence of the *cag*PAI determines the extent of gastric inflammation and subsequent disease severity in patients [55] and in suitable animal models, for instance the Mongolian gerbil [56,57]. The secretion system is expressed in the stomach [58,59] and can translocate various molecules into gastric epithelial cells, including bacterial DNA [40] or the NOD1 ligand and peptidoglycan metabolite ieDAP [41]. Interestingly, live *H. pylori* seem to suppress the important innate DNA and RNA sensory systems cGAS-STING and RIG-I [60].

Recently, we and other researchers contributed to the discovery and characterization of a novel activation mode and bacterial MAMP, which plays a major role for *H. pylori* interaction with the human host and human gastric epithelial cell signaling. This important *H pylori* activation is mediated by bacterial LPS inner core precursor molecules including heptose metabolites [23,61,62]. The epithelial cell activation by those bacteria-specific small metabolites is strain-variable and modulated by *H. pylori* [23,24,41,45,61,63]. A substantial portion of early NF-κB activation by *cag*PAI-positive *H. pylori* appears to be due to the CagT4SS-mediated transport of heptose metabolites into the gastric epithelial cells [61]. At later time points of cell interaction, other innate signaling pathways, such as NOD1- or NOD2-mediated ones, seem to become more prominent [23]. *H. pylori* appears to use mainly its Cag T4SS to mediate the direct cell transfer of heptoses [22,23,61], while free heptoses are released only sparsely into the environment by live *H. pylori*.

Interestingly, other bacteria, such as pathogenic *Enterobacteriaceae* (*Shigella*, *Yersinia*, *Salmonella*), may enlist their Type III secretion systems (T3SS) for a similar purpose [64], namely transporting bacterial heptose metabolites into eukaryotic cells. Hence, heptose activation seems to be a very common host activation mechanism by pathogenic bacteria. Type IV secretion systems, similar as known for T3SS, can transport both proteins and metabolites, for the purpose of dramatically influencing innate cell-autonomous signaling and activation in eukaryotic cells.

Although there are questions remaining to be investigated in (native) gastric epithelial cells, knowledge in this research area is comparably far advanced. There is still much less information available on the specific mechanisms of interaction and crosstalk of *H. pylori* with cells of the myeloid lineages in *Homo sapiens*, for instance, the phagocytic and antigen-presenting cells. Similar methodological limitations as for the epithelial cells apply to the study of native phagocytic cells *in vivo* during human infection. Thus, one purpose of this review is to summarize the current knowledge about how and by which specific factors *H. pylori* can interact with human macrophages and related myeloid cell types *in vitro* or in its natural niche in the human stomach, and to generate a knowledge base in order to define which further lines of research in this area are required. It is always important to consider whether reported evidence was gathered in mouse or in human experimental systems, since *H. pylori* are superbly adapted to humans.

Current knowledge on the influence of *H. pylori* on cells of different myeloid lineages and antigen-presenting cells *in vivo* and *in vitro*

In contrast to epithelial cells, the role of phagocytic cells during infection is commonly assigned to directly eliminate the pathogens locally and to translate the immediate danger signals to adaptive immune cells such as T-cells and antibody-producing B-cells. *H. pylori*, given the chronic nature of its infection in the human stomach, supposedly has evolved to evade mechanism of diverse phagocytes. But do we have sufficient evidence for this hypothesis? Macrophages, monocytes, immigrating neutrophils and resident DCs, were identified in the *H. pylori*-infected mucosa [65,66]. Different approaches have been followed as reported in prior publications to identify the phagocytic cell populations and their interactions with *H. pylori* in the stomach: cells have been isolated directly from biopsies of infected human individuals [66], and the phagocytic cell types contained in the biopsies were sorted and characterized by surface markers. Neutrophils and macrophages are present locally, and DCs that are present at the site of infection partially also arise from immature monocytes [67]. In addition to phagocytosis and antigen presentation to induce the adaptive immunity (T-cell and B-cell activation), neutrophils can degranulate to release microbicidal molecules and generate neutrophil extracellular traps (NETs) to trap and eliminate some pathogens [68]. Concerning *H. pylori*-phagocyte interactions, the modification of some of such processes can be suspected or has already been discerned.

Macrophages

Macrophages, which differentiate from monocytes, can develop, among other outcomes, into the proinflammatory M1 phagocyte type or the rather immune-inhibitory M2 type, or mixtures thereof [69–72], which can be differentiated by phenotype and transcriptomic signature. In general terms, they are supposed to phagocytose the pathogen, and subsequently, M1 macrophages present antigens to lymphocytes (T-cells). Associated cytokine secretion concomitantly recruits other immune cells to the vicinity of the infection and the local lymphatic tissues [66,73]. In mice, *H. pylori* infection was associated with an M1 macrophage phenotype *in vivo* [74,75]. Quiding-Järbrink and colleagues also compared mouse with human gastric macrophages under the influence of *H. pylori*. While M1 macrophage type was present in atrophic human gastritis under *H. pylori* infection, a premalignant lesion, local macrophages in less symptomatic *H. pylori*-positive patients were of a mixed M1/M2 type [75]. Likewise, Fehlings and colleagues [66] found both M1 and M2 polarized macrophages in gastric biopsies of *H. pylori*-infected patients. However, the biopsy approach is tedious and inaccurate, due to the limited availability of tissue samples, small, locally restricted, sampling size, and lack of information about coinfections and microbiota effects. Since a Th1 or Th17 T-cell response predominates during the chronic *H. pylori* infection *in vivo*, the assumption is that macrophages present locally during the infection are mainly of the M1 type, which is more proinflammatory and produces less of the tolerogenic cytokine IL-10. This remains to be proven under different infection and bacterial strain settings and in different individuals and disease stages. It was not well characterized before, whether *H. pylori* induces a rather M1-like or M2-like macrophage differentiation from monocytes *in vitro*. Fehlings and colleagues [66], using such an approach for primary mouse macrophages (blood- or bone marrow-derived) found that *H. pylori* differentially influences the response of predifferentiated M1 or M2 macrophages. Other studies for the characterization of *H. pylori* interaction were making use of immortalized monocytic or macrophage cell lines of human or mouse origin in cell culture, such as Thp-1 (human), U937 (human) or Raw294 (mouse), in cell culture. Such model systems were primarily applied to investigate phagocytosis of the bacteria [76]. Phagocytosis and phagosome maturation of macrophage-like cells or primary human macrophages are inhibited by *H. pylori* [76–78]. Autophagy seems to play a very minor role as a defence mechanism against *H. pylori* in macrophages [79]. However, the understanding of how *H. pylori* adheres to and is taken up by phagocytic cells and by which factors the bacteria are influencing their interaction phenotype or activities is still very limited. Divergent data were reported on the role of the *cag*PAI in activating phagocytes or altering phagocytic activities during bacterial encounter [74,77,80]. The T4SS is less active in mouse, probably due to human-specific mechanisms and cofactors. Koch and colleagues [74] reported, using primary macrophages collected from mouse bone marrow, that the *cag*PAI influences the activation of macrophages, independent of Toll-like receptor pathways. Transcriptome changes particularly in TLR signaling-impaired macrophages (Myd88-TRIF-negative) in mouse background showed a signature of CagT4SS-dependent but CagA-independent transcript activation. For those questions, more detailed data about mouse models are available, while data in human systems are lacking. We are still missing mechanistic details of the signaling pathways in both species. Our lab recently examined the innate immune activities of *H. pylori* on human cell culture monocyte/macrophages (Thp-1) and primary human monocyte-derived macrophages [81]. We demonstrated that *H. pylori*-activated human macrophages exhibit a strongly proinflammatory cytokine signature, including high IL-8 secretion, to *H. pylori* and heptose metabolites, collected from *H. pylori* or synthesized *in vitro*. In addition, using detailed transcriptome analyses, we determined that monocyte-like cells rather polarized into an M1 proinflammatory macrophage phenotype in this uniform cell culture model after activation with pure soluble heptose metabolites or with *H. pylori* strains. LPS heptose-biosynthesis-deficient bacteria did not activate the

macrophages to the same extent. Likewise, *H. pylori* lacking the CagT4SS were less efficient to mature and activate monocytes or macrophages, demonstrating that an active *cag*PAI is important ([81]; and see below). These results complement and support the previous *in vivo* human and mouse data [75], without being able to mirror all aspects of a complex organismal model. Similar to epithelial cell effects, this work established a major role of heptose metabolites and their translocation by the CagT4SS on the innate activation of human monocytes and macrophages. TLR2 activation also plays a very important role in the innate activation of cultured and primary human macrophages by *H. pylori*, (Faass and Josenhans, partially unpublished), while it was known early on that TLR4 is only weakly addressed by *H. pylori* lipidA/LPS in human macrophages [82].

Based on this knowledge, [83] recently showed that the *H. pylori* LPS heptose metabolite ADP-heptose, exhibiting an important role in monocyte and macrophage activation [81], is also the decisive factor reducing the expression of antigen-presenting surface molecules of macrophages. Pure ADP-heptose, live bacteria, and heptose-enriched *H. pylori* lysates lead to the upregulation of a noncoding miRNA, miR146b, in macrophages that negatively affects the expression of CIITA (HLA-II protein expression transactivator), while heptose-deficient *H. pylori* did not. HLA-II expression and surface display by the cells were both hampered, which reduced the effect of activated macrophages to interact with and present antigen to T-cells [83]. Hence, from our recent data, it seems very likely that *H. pylori*, by means of LPS heptose metabolite production, negatively influences antigen presentation despite high macrophage cell activation, and thereby hinders signal transfer from the innate to the adaptive immune system. These findings so far provide a controversial picture showing proinflammatory and immune evasive downstream processes in phagocytic cells upon *H. pylori* exposure. Part of the difficulties encountered by us and others to obtain a clear picture of phagocyte responses may relate to the influence of opposite or counterbalancing, and even strain-specifically expressed, factors of *H. pylori*. Such factors can act on the target cells simultaneously, but may also be regulated by the bacteria in a strain-dependent and context-dependent manner (Hauke and Josenhans, own unpublished data). ROS production seems to be less prominently induced in human in comparison to mouse macrophages upon exposure to *H. pylori* [84]. The reason for this difference is unknown and may imply some inhibitory bacterial factors.

Dendritic cells

DCs raise a cell-autonomous immune response to infection, similar as macrophages [85]. In addition, they are essential to present antigen to T-cells and B-cells [86,87]. With respect to the downstream effects resulting from the interaction of *H. pylori* with such professional phagocytes, researchers detected a balance between immune-stimulating or -inhibiting activities: Firstly, the phagocytic capacity of human primary dendritic cells appears not to be impaired after coincubation with live *H. pylori* [66], which seems to be uncommon with respect to the initial activation of dendritic cells [86]. In addition to high amounts of proinflammatory cytokines that are secreted by *H. pylori*-infected primary human dendritic cells, the macrophage inhibitory factor was shown to be secreted in significantly reduced amounts, indicating the potency to boost an excessive proinflammatory response [66] in the infected gastric environment of human patients. An essential role of NLRP3 activation by *H. pylori* in DCs was postulated [42]. However, the latter study was performed entirely in mouse DCs, calling for a verification in a human test system. [43] determined, also for mouse DC, that TLR2 is a major, and TLR4 a minor PRR being able to recognize *H. pylori* and mount a downstream innate immune response. In addition, they reported for the first time that the intra-endocytic PRR TLR9 senses *H. pylori* DNA in phagocytes. This result was later confirmed in a human epithelial cell culture model, where the authors, in addition, identified a contribution of the CagT4SS in DNA transport and innate cell activation [40]. Mechanistic studies in human primary DCs will be facilitated in the future by improved CRISPR-mediated k/o technology, for instance, to inactivate PRR genes.

Neutrophils

Neutrophils are very important mediators in the *H. pylori*-infected stomach. Firstly, they were identified early on as a hallmark of chronic active gastritis, inherent in all infected persons albeit to a different extent [88,89]. Chronic gastritis provides a sustained local proinflammatory response over time [65,90,91]. Secondly, neutrophils are supposed to be highly effective, specifically against extracellular pathogenic bacteria, since they have specific mechanisms set up against those. The most important ones known are oxidative burst, NET formation, phagocytosis, and autophagy [68,92–94]. Neutrophils are recruited to *H. pylori*-infected sites in the stomach, chemotaxing to high local IL-8. Despite this massive influx of neutrophils during acute and chronic infection, the bacteria are not effectively removed from the human stomach. This raises the question how neutrophils are activated by *H. pylori*, and whether and how the bacteria may suppress or evade effective neutrophil responses.

Concerning PRR recognition of *H. pylori* by human neutrophils, [95] reported that human neutrophil TLR9 seems to be addressed by *H. pylori* lysates, but with a much weaker response than for whole bacteria. We have little knowledge on the activation of other neutrophil-expressed PRR by *H. pylori*. TLR2 and TLR5 are being

cell-autonomously downregulated in primary human neutrophils by *H. pylori* exposure [96], while TLR9 was upregulated. With respect to inflammasome activation and IL-1β production, studies reported early on that, while NLRP3 and ASC are induced in human primary neutrophils by *H. pylori*, and IL-1β is being produced subsequently, neither the bacterial T4SS nor TLR2 or TLR4 were contributing to this effect [97]. More recently, it was reported [98] that NLRP3 inflammasome activation, by way of the bacterial T4SS and flagellin, is very important during the infection of neutrophils. This study confirms first of all that the NLRP3 inflammasome is crucial in inducing IL-1β in human and mouse neutrophils exposed to *H. pylori*. The authors also find that the CagT4SS seems to be primarily involved in signal 2, leading to the release of mature IL-1β, in both mouse and human neutrophils, and less in the induction of priming signal 1. TLR2 activation seems a major priming signal (signal 1) for *H. pylori*-induced IL-1β secretion (mouse). Lastly, bacterial flagellin FlaA, by way of motility, but not through TLR5 or NLRC4 PRRs, contributed to IL-1β production and release in both mouse and human neutrophils, probably by increasing bacteria-cell contact [98]. However, this study, with a focus on inflammasome activation in neutrophils, has to be interpreted with some caution, as the k/o studies were exclusively performed in mice. Broad knowledge was accumulated on the human neutrophil activation protein (HP-NAP), produced and secreted by *H. pylori*. The most prominent HP-NAP effects reported to date include to directly activate neutrophils (possibly via TLR2) and monocytes, and to act in an immunomodulatory manner. We will not explore this factor in detail here, since recent reviews have covered the existing literature extensively [99–101]. Our laboratory recently found (own unpublished results) that ADP-heptose metabolites, including such metabolites produced by *H. pylori*, which also activate other phagocytic cell types avidly, seem to be extremely active on neutrophils.

On the other hand, *H. pylori*-mediated mechanisms that impair human neutrophil function have been reported recently [102]. They demonstrated, in human patients, human cell culture, and in a mouse model, that cathepsin C (CtsC), a dipeptidyl peptidase I, which is important for the neutrophil proteolytic and bactericidal activity, is inhibited by *H. pylori*. This effect seems to occur mostly in gastric epithelial cells of human and mouse and thereby indirectly leads to impaired killing efficacy in neutrophils (less ROS production and reduced phagocytosis), and to less efficient clearance of *H. pylori* in a mouse model. NF-κB p65 activation in neutrophils seems to be required for the phenotype. They did not investigate which bacterial mechanism impacts on CtsC, nor if CtsC expression is also reduced in neutrophils themselves in their model. *H. pylori* also seem to counteract the neutrophil oxidative burst intracellularly, for instance, by changing the trafficking of NADPH oxidase towards the plasma membrane and preventing its activity in endosomal/phagosomal compartments [103]. Neutrophil chemotaxis and cell motility were also impeded by *H. pylori* [104]. The mechanisms were not explored further to date. Human neutrophil phagocytosis also was impaired by *H. pylori* in culture, namely via its Cag T4SS and effector CagA [105]. Bacterial outer membrane protein HopQ-Ceacam interactions, which, for instance, are not as active in mouse cells as in humans, but are essential for CagA translocation by the CagT4SS, contribute to impeding *H. pylori* phagocytosis by human neutrophils [105]. Live, adherent *H.pylori* induces subtype differentiation of human neutrophils [106], most likely by cell-autonomous PRR ligation. The same researchers also showed that *H. pylori* infects human neutrophils directly, by lectinophagocytosis, and leads to hypersegmentation of nuclei and increased neutrophil survival. The infected neutrophils also did not release DNA, which implies the absence of NET formation [106].

Role of soluble *H. pylori* bacterial factors and proteins in the interaction with and activation of phagocytic cells

The Cag T4SS plays an important role not only for *H. pylori* interaction with epithelial cells but also with phagocytic cells. Concerning important soluble factors transported by the T4SS, we have recently characterized in detail that Gram-negative bacterial heptose metabolites, also produced by *H. pylori*, are major innate mediators for the activation and maturation of macrophage-like cells and neutrophils ([81] and own unpublished results). They act via the recently defined innate ALPK1-TIFA axis [107–109]. The CagT4SS also transports the oncogene protein CagA into epithelial cells [110–112] and macrophages, where it can facilitate phagocyte uptake [77]. CagA can be degraded by autophagy [113], and influences cell morphology and intracellular signaling [112,114].

The immune modulatory pore-forming *H. pylori* toxin-like protein, VacA, autotransports itself into gastric epithelial and other cells and causes modification of intracellular membrane trafficking [115], besides being involved in proapoptotic signaling [116]. VacA is able to inhibit the activation and proliferation of T-cells via suppression of NFAT activity, which results in a resistance against immune clearance and may contribute to the frequently life-long persistence of *H. pylori* [117,118]. VacA functions in diverse human cell types are reviewed in detail by [81,119]. Briefly, VacA influences a variety of antigen-presenting cells, inhibiting proliferation of primary human B-cells, and impairing antigen presentation [119]. In macrophages, VacA can lead to apoptosis [120,121]. When VacA is transported into phagosomes from internalized VacA- and *cag*PAI-

positive *H. pylori*, phagosome maturation is delayed, endosome-lysosome fusion is inhibited, and phagosome-phagosome fusion, resulting in megasomes containing multiple vital bacteria, is enhanced [122,123]. Intracellular killing of the bacteria is inhibited in those models [124,125], which corresponds to earlier data [80]. For DCs, VacA is an important factor that mediates a mostly tolerogenic phenotype upon *H. pylori* infection, thereby promoting persistence [126]. Oertli and coworkers [127] demonstrated that VacA-positive *H. pylori* effectively prevented murine BM-DCs from maturation and reduced the expression of T-cell activation markers. In conclusion, although professional phagocytes and antigen-presenting cells, including B-cells, have been somewhat overlooked as potential VacA targets, a considerable amount of evidence in support of strong immunosuppressive effects on these cell types exists [124].

Urease is one of the most important soluble colonization and virulence proteins of *H. pylori*, locally reducing extracellular stomach acidity and balancing intracytoplasmic bacterial pH [128], thereby supporting bacterial colonization of the acidic stomach [129–131]. It is also a potent macrophage chemoattractant, which modulates phagocytosis by delaying opsonization [132]. Urease plays a key role in affecting phagosome pH and megasome formation [133] and as such, is essential for *H. pylori* survival in macrophages. NapA or HP-NAP, a DNA binding, bacterio-ferritin-like oligomeric secreted protein [99,101] was highlighted above as a major activator of monocytes and neutrophils, able to modulate both innate and adaptive immune responses. Further interaction modes of *H. pylori* soluble factors and effectors with myeloid cells await discovery.

Surface and receptor interaction of *H. pylori* with phagocytic cell types, bacterial adherence, and morphological effects

While it is well known how *H. pylori* interacts with and activates gastric epithelial cells via its adhesins, mainly via BabA ($Le^{b,}$ blood group H antigen) binding and SabA (sialylated sugars such as glycosphingolipids), the molecular mechanisms of its primary surface interaction with lymphoid and phagocytic cells are less well understood. Different receptor classes are available for interactions with bacteria. Extracellular innate immune PRR receptors, including the Toll-like receptors, are variably present on phagocytic cells and may bind bacteria or bacterial molecules; however, they usually contribute less to the bacterial tight adherence phenotypes. *H. pylori* binds to phagocytic cells less avidly than to gastric epithelial cells [77,134]. Causes for the low binding of *H. pylori* to phagocytic cells are less clear, but may also affect modes of cell activation and phagocytic activity [76,80]. Phagocyte surface glycans may directly bind to bacterial surface adhesins. The major *H. pylori* adhesin SabA, which binds sialylated glycan structures, is involved in the binding and activation of human neutrophils [134]. Phagocytes are rich in cell surface PRR such as C-type lectins (CTL) [135]. Membrane-integrated or soluble CTL contribute to proinflammatory and tolerogenic (dampening) cell signaling [135]. Phagocytosis of *H. pylori* by neutrophils [136,137] is attributed to lectin interactions (lectinophagocytosis; [106]).

Prominent phagocyte-lectin interactions are characterized for *H. pylori* with the CTLs MINCLE, DC-SIGN, galectins, and mannose-binding lectins [138–142]. DC-SIGN is expressed on DCs, and its regulation influences DC potency to control immunity [143], rather contributing to tolerogenic signaling, since its glycan ligands are self-like glycans. DC-SIGN [138,144] binds sialic acids, which are variably present on the surface of *H. pylori* [145]. *H. pylori* performs molecular mimicry using sialylated surface sugars such as sialylated $Lewis^X$, Le^Y, and fucose in its LPS outer chains [146]. DC-SIGN interactions (on DCs) with *H. pylori*, due to fucose interaction, lead to dampened responses of phagocytes [147] and to the selective activation and proliferation of immunosuppressive regulatory T-cells, which suppress gastric inflammation [148]. Chmiela et al.[149] demonstrated that sialic acid-dependent and sialic acid-independent adhesins support *H. pylori* binding to macrophages. These interactions, despite being of minor importance for tight and permanent bacterial binding, are very important for immunomodulatory activities imposed by the bacteria. MINCLE, a fucose-binding CTL, was shown to contribute to the activation of bone marrow-derived macrophages and DCs (mouse) by binding *H. pylori* membrane cholesteryl-glycosides [150,151]. This interaction may subsequently activate innate NKT cells [152]. The enzymatic glucosylation of cholesterol by *H. pylori* was determined to inhibit phagocytosis (mouse macrophages) and T-cell presentation [153]. Bacterial cholesterol glycosylation, possibly by DC-SIGN interaction, may contribute to inhibition of phagocytosis and phagosome maturation ([154]; mouse cultured macrophages). It is assumed that this interaction also occurs in human. Lectinophagocytosis by phagocytes [106] can also be promoted by the above-mentioned lectin interactions.

Variation of bacteria in immune-modulatory traits

H. pylori is one of the most variable pathogenic bacteria, displaying a unique genetic fingerprint in each strain [155] and even a strong intrapopulation diversification in one single patient stomach [156]. This led us to hypothesize that each strain effectuates a different cell-autonomous immune activation and even has possibilities to individually vary its interaction with human cells. This is an area where much remains to be

discovered and we will not discuss here the individual variation on the human side or coevolution. What is currently known for bacterial variability in relevant interaction traits? Strongly strain-variable surface factors, for instance of the outer membrane family (BabA, HopZ; [37]) and the CagT4SS [33,157], can influence bacterial cell adherence, cell activation and bacterial motility, and thereby broadly the cell-autonomous innate response. Extensive evidence for strain variation in innate activation potential was gained in a comparative study within a global strain collection [33]. Much of this variation seems to be due to CagT4SS-mediated transport of bacterial metabolites [23,61]. In addition, surface variation of CagT4SS proteins such as CagY was found to modulate the activity of the CagT4SS [158]. Such traits can even switch on or off during an early chronic infection [159]. Our most recent data indicate that *H. pylori* strains harbor individual regulation and variation mechanisms in producing innate active glycan metabolites transported via the CagT4SS (Hauke and Josenhans, own unpublished data). Such variation might even extend to environmental regulation of gene expression and CagT4SS transport activity. Since other relevant *H. pylori* traits, alleles, and host-directed proteins (see above chapters on soluble factors) have shown considerable strain variation, the list of potential mechanisms to mine in the future for strain variation in host modulation seems vast.

Conclusions and outlook

We can safely conclude at this point in time already from the existing literature that *H. pylori* interacts in a specific and strain-specific manner with epithelial and phagocytic cells, including some overlap such as the importance of heptose metabolite transport and innate signaling. *H. pylori* also appears to use its CagT4SS for fine-tuning these interactions, strain-specifically, over time, and in all so-far characterized cell types, in particular in monocytes-macrophages and neutrophils. *H. pylori* has an ambiguous behavior towards its host, indicating a balance between proinflammatory responses, tolerogenic and immunosuppressive/-evasive mechanisms in various host cell types involved during an active *H. pylori*-driven gastric infection and inflammation. Induction of those responses may also be induced strain-specifically and modulated dynamically by the bacteria, in response to cell reaction and environment.

Despite the knowledge accumulated about immune activation of human gastric epithelial cells by *H. pylori* and their possible evasion/suppression mechanisms, it is still understudied how they are addressed and manipulated by the bacteria, mainly due to a dearth of primary materials from patients [28,160]. Primary cells should be used to complement cell culture models [43,161,162]. We still lack major information on inter-strain variation and the time course of bacterial activities in various models and cell types, which should encourage the future mining of variable bacterial factors. So far, innate heptose activation has been studied mainly at early interaction time points in human cell models, but may play a role continuously during all stages of the infection, since it can be specifically produced and replenished 'upon demand' by the bacteria (own unpublished results). Organoid models have already been developed for life-like multicellular assemblies of various cell types, co-occurring in the gastric crypts [10,163,164]. Coincubation of gastric organoids [20,165,166] with *H. pylori* strains has already yielded novel insights [9,10,167,168,169]. However, the interplay between tissue/somatic and immune cells and the bacterial pathogens in such multicellular models still needs further model adjustments. *In vivo* models for *H. pylori* infection are particularly difficult to develop for this human-specific pathogen. A variety of animals have been successfully infected with *H. pylori*, including mice, Mongolian gerbils, nonhuman primates [57,170–172]. However, each of the models has severe limitations and drawbacks, for example, the function of the CagT4SS, proinflammatory and cancerogenic mechanisms seem to have host species-specific aspects. All those considerations make it difficult to extrapolate the results from animal studies to humans [173]. Taken together, improvements of model systems or development of novel models should be urgently followed up.

Remaining questions, for example, about known and unknown bacterial soluble products, metabolites and other factors modulating the innate activation stay on the wish list for further scientific investigations. Work in human primary phagocytes will be supported in the near future by sophisticated multiple k/o or knock-in techniques adapted to primary cells. A prophylactic and therapeutic vaccine would be desirable to combat *H. pylori*-mediated diseases, but so far, vaccine approaches *in vivo* in humans have worked at best poorly [174,175]. Specific vaccine development against *H. pylori* is certainly hampered by its incompletely understood immune evasive and modulatory mechanisms and a lack of knowledge of their interaction with certain immune cell types. For these reasons, we consider it very helpful to gather more in-depth information about the specific interaction of *H. pylori* with human phagocytic cell types and the manipulation of their function.

Conflict of interest statement

The authors indicate no conflict of interest.

Acknowledgements

We are grateful for funding of our summarized and ongoing research in this area by the Center grant CRC 900 (German Research Foundation, DFG, Germany, project no. 158989968), in particular project B6 to CJ, the DFG single grant project JO 344/5-1 to CJ, and the German Center of Infection

Research (DZIF), Germany, projects 06.809 and 06.820, to CJ. LF and MH were additionally supported by the graduate program "Infection Research on Human Pathogens at MvPI." We thank all past and present lab members of the Josenhans lab for constructive comments, discussions, and contributions to the research projects.

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