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Immune Cell Signaling by *Helicobacter pylori*: Impact on Gastric Pathology

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Abstract

Helicobacter pylori represents a highly successful colonizer of the human stomach. Infections with this Gram-negative bacterium can persist lifelong, and although in the majority of cases colonization is asymptomatic, it can trigger pathologies ranging from chronic gastritis and peptic ulceration to gastric cancer. The interaction of the bacteria with the human host modulates immune responses in different ways to enable bacterial survival and persistence. *H. pylori* uses various pathogenicity-associated factors such as VacA, NapA, CGT, GGT, lipopolysaccharide, peptidoglycan, heptose 1,7-bisphosphate, ADP-heptose, cholesterol glucosides, urease and a type IV secretion system for controlling immune signaling and cellular functions. It appears that *H. pylori* manipulates multiple extracellular immune receptors such as integrin-β_2 (CD18), EGFR, CD74, CD300E, DC-SIGN, MINCLE, TRPM2, T-cell and Toll-like receptors as well as a number of intracellular receptors including NLRP3, NOD1, NOD2, TIFA and ALPK1. Consequently, downstream signaling pathways are hijacked, inducing tolerogenic dendritic cells, inhibiting effector T cell responses and changing the gastrointestinal microbiota. Here, we discuss in detail the interplay of bacterial factors with multiple immunoregulatory cells and summarize the main immune evasion and persistence strategies employed by *H. pylori*.

Keywords

T4SS · TLR · PAMP · PRR · Inflammasome

1 Introduction

Hallmarks of microbial infections are inflammation and subsequent changes in the affected tissue. Generally, most infections are cleared by the host immune system through innate and adaptive responses. Microbial invasion can be detected by a plethora of factors belonging to the innate immune machinery. The well-studied pattern recognition receptors (PRRs) detect pathogen associated molecular patterns (PAMPs) and this interaction leads to various arms of signal transduction to produce a timely response by the immune system to control the infection (Takeuchi and Akira 2010). In addition, inherent signals of anti-inflammation are needed to subsequently down-regulate the immune activity and avoid unnecessary damage to host tissue. Many pathogens have developed strategies to evade host immune responses to variable degrees. For instance, the untimely induction of both pro- and

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anti-inflammatory responses will jeopardize the control of infection and avoid a return to homeostasis. *H. pylori* is an example of a pathogen that effectively manipulates the host's immune response upon infection. It colonizes the human gastric mucosa and is associated with gastritis, peptic ulcer and gastric cancer. When present, *H. pylori* is effectively detected by the host innate immune system, which in response produces pro- and anti-inflammatory cytokines and other inflammatory mediators (White et al. 2015). These responses ought to be sufficient to result in adaptive immunity against this pathogen, but they cannot effectively clear the infection, which allows *H. pylori* to colonize an individual from childhood (when most primary infections occur) to last an entire life span.

In 10–20% of cases, infection with *H. pylori* is associated with the development of peptic ulcers and about 1–2% develop gastric cancer or gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Wroblewski et al. 2010; Bauer and Meyer 2011). A major risk factor for these *H. pylori*-associated diseases is local chronic gastric inflammation in response to colonization (Dunn et al. 1997; White et al. 2015; Gobert and Wilson 2016). The immune response during *H. pylori* infection is characterized by the infiltration of several types of immune cells, for instance anti-*H. pylori* T-cells were found in the gastric mucosa of infected individuals (D'Elios et al. 1997). The prolonged pathogenesis by this bacterium is based on its adaptation to and survival in the harsh conditions of the stomach, to which *H. pylori* responds by differential regulation of its gene expression during colonization (Wang and Maier 2015; Gieseler et al. 2005). Analyzing the bacterial gene expression of *H. pylori* derived from mouse stomachs or infected cultured murine cells showed an up-regulation of the cytotoxin-associated gene A (*cagA*) encoding the CagA effector protein and of *vacA* encoding the vacuolating toxin VacA (Singh et al. 2012). Both gene products are well-characterized virulence factors of *H. pylori* (Posselt et al. 2013). CagA, which was amongst the first virulence factors to be discovered for this pathogen, can hamper the maturation of dendritic cells (DCs), as demonstrated using both cultured human cell lines and a mouse model, suggesting it employs an immune regulatory effect (Tanaka et al. 2010; Käbisch et al. 2014). Effects on cellular vacuolation, apoptosis or immune cell inhibition are described for VacA, while the *cag* pathogenicity island (PAI), encoding a type IV secretion system (T4SS), is crucial for delivery of CagA across the bacterial membrane into the host cells (Backert et al. 2011; Bridge and Merrell 2013; Naumann et al. 2017). It appears that the inflammatory response during *H. pylori* infection is mainly controlled by the *cag*PAI, in line with the observation that *cag*PAI-positive strains (which have been designated as type-I) are more virulent compared to *cag*PAI-negative (type-II) isolates (Tegtmeyer et al. 2011). Later studies showed that only strains positive for CagA and VacA are able to drive immune cell tolerance during infection, presumably to promote chronic persistence of the pathogen (Oertli et al. 2013; Käbisch et al. 2014). Studies investigating the impact of *H. pylori* have shown that VacA can directly interact with T-cells, B-cells, monocytes and macrophages, which trigger both immune stimulatory and suppressive activities (Boncristiano et al. 2003; Gebert et al. 2003; Singh et al. 2012).

In addition, *H. pylori* is able to weaken the gastric epithelial barrier function and can induce epithelial apoptosis (Backert et al. 2017, 2018). Disruption of the epithelial integrity leads to increased amounts of bacterial virulence factors in the lamina propria, where they can interact with immune cells (Mai et al. 1991, 1992). Disrupted epithelial barrier function and epithelial apoptosis can be induced by VacA, but also by the secreted enzyme γ-glutamyl transpeptidase (GGT), which also contributes to the virulence of *H. pylori* (Cover et al. 2003; Shibayama et al. 2003). Besides its importance during colonization as demonstrated by *in vivo* models, GGT was further shown to promote cell cycle arrest, increase production of reactive oxygen species and induce the secretion of inflammatory cytokines leading to apoptosis and necrosis of gastric epithelial cells (Chevalier et al. 1999; McGovern et al. 2001; Oertli et al. 2013). The enzyme GGT, which is highly conserved between *H. pylori* strains,

catalyzes the hydrolysis of glutamine to glutamate and ammonia and further the hydrolysis and transpeptidation of various γ-glutamyl compounds (Shibayama et al. 2007; Song et al. 2011). Apart from their direct functional effect, virulence factors can also modulate the cellular signaling and homeostasis. For example, cholesterol, a common constituent of the host cellular membranes, is extracted and converted to glucosides by cholesterol-α-glucosyltransferase (CGT) of *H. pylori*, which prevents phagocytosis and subsequent antigen presentation. Furthermore, cholesterol depletion dampened specific cellular signaling like interferon gamma (IFNγ) by depleting its receptors from lipid rafts (Wunder et al. 2006; Lai et al. 2008, 2011; Morey et al. 2018). Antigen presentation was tightly regulated at different levels by different mechanisms like interference on phagosome maturation, downregulated expression of antigen presenting MHC (major histocompatibility complex) molecules, co-stimulatory factors, and T-cell differentiation to regulatory phenotypes (Molinari et al. 1998; Ramarao et al. 2000; Allen 2007; Beswick et al. 2007; Wang et al. 2010). The interaction of *H. pylori* proteins like urease subunit B (UreB), heat shock protein 60 (Hsp60) and neutrophil activating protein A (NapA) with Toll-like-receptor 2 (TLR2) resulted in varied responses (Amedei et al. 2006; Zhao et al. 2007; Koch et al. 2015). Moreover, common PAMPs like lipopolysaccharide (LPS), peptidoglycan, DNA and RNA of *H. pylori* were shown to induce various pro- and anti-inflammatory signals through their respective PRRs (Ishihara et al. 2004; Viala et al. 2004; Allison et al. 2009; Rad et al. 2007; Nagashima et al. 2015). *H. pylori* infection also regulates the inflammasome for the secretion of cytokines interleukin 1β (IL-1β) and IL-18, which ultimately favors bacterial survival and persistence (Kim et al. 2013; Koch et al. 2015; Ng et al. 2016; Pachathundikandi and Backert 2018). The induction of pro- and anti-inflammatory states in this infection skewed for both reduction in bacterial colonization and immune-pathologies, but never resulted in resolution of inflammation without prophylactic methods (Garhart et al. 2002; Matsumoto et al. 2005; Kao et al. 2010; Quiding-Järbrink et al. 2010; Cook et al. 2014). Here we review the overall interplay of these various *H. pylori* factors with the host's immune system.

2 The Role of *H. pylori* GGT on Immune Tolerance

H. pylori GGT induces immune tolerance through altering DC processes, and GGT enzymatic activity is needed for this immune regulation, as was first demonstrated in infected mice (Oertli et al. 2013). Subsequently, by infecting human DCs with *H. pylori* Käbisch and co-workers (2016) showed that GGT promotes the progression of naïve T-cells to regulatory T-cells. As a result of the enzyme's activity, levels of glutamate increase in the stomach and these promote the activation of glutamate receptors that are expressed on DCs; this induces immune tolerance during *H. pylori* infection (Shibayama et al. 2007; Käbisch et al. 2016). Recently, Wüstner et al. (2015) have shown that activated T-cells are highly sensitive to glutamine concentrations in the extracellular space. In addition, previous studies have identified an inhibitory effect of insufficient glutamine levels on T-cell proliferation (Yaqoob and Calder 1997). Moreover, the expression of transcription factors that stimulate T-cell receptor signaling and influence their differentiation and expansion is decreased in the presence of active GGT, suggesting a hampering effect of *H. pylori* GGT on the activation and proliferation of these immune cells (Man et al. 2013; Yao et al. 2013).

Käbisch and co-workers (2016) showed, by infecting human DCs with *H. pylori* wild-type and an isogenic Δ*ggt* deletion mutant, respectively, that *H. pylori* can suppress the secretion of IL-6. Moreover, using glutamate receptor inhibitors it was shown that glutamate might have a regulatory impact on IL-6 secretion, influencing downstream T-cell responses to the pathogen. Conversely, insufficient glutamine levels resulting from inactive GGT might affect the production of IL-2 or IFNγ, indicating that by

modulating its GGT activity *H. pylori* can actively influence the secretion of specific cytokines (Carr et al. 2010). This ability is not restricted to *H. pylori*; during infection with *Helicobacter suis,* supplementation with glutamate showed protection against bacterial-induced pathologies and suppression of inflammatory cell infiltration, underlying the importance of GGT in immune regulation (De Bruyne et al. 2016).

3 Interference of VacA with T-Cell Receptor/IL-2 and Nuclear Factor of Activated T-Cells (NFAT) Signaling

Although both VacA and GGT act on T-cells and are involved in inducing tolerance, they accomplish this via entirely different pathways (Fig. 1). Various studies have shown that VacA hampers the proliferation of T-cells, for which multiple mechanisms have been proposed (Boncristiano et al. 2003; Gebert et al. 2003; Sundrud et al. 2004). Differential binding and uptake of VacA in epithelial and immune cells was described more than 10 years ago (Gauthier et al. 2005). In immunoprecipitation studies, the CD18 receptor of human T-cells was identified to be targeted by VacA and seemed to act as a receptor or co-receptor for the toxin (Sewald et al. 2008). Indeed, in human primary T-cells, CD18 expression is essential for the uptake of VacA and subsequent cellular effects. However, a determinative difference between the VacA internalization exists between human and murine T-cells, indicating that specific pathways are responsible for the VacA-dependent effects in the human host (Sewald et al. 2008).

In general, T-cell receptor (TCR) activation by antigen-presenting cells, together with co-stimulation, is the first step for a proper T-cell response (Zheng et al. 2008; Smith-Garvin et al. 2009; Brownlie and Zamoyska 2013). As summarized by others, TCR engagement causes several phosphorylation events leading to the recruitment of effector proteins (Roifman and Grunebaum 2013). This results in actin cytoskeleton rearrangements, activation of Ras GTPase, and calcium mobilization (Nika et al. 2010; Roifman and Grunebaum 2013). Subsequently, various transcription factors become activated, such as nuclear factor of activated T-cells (NFAT) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which initiate the transcription and secretion of pro-inflammatory or pleiotropic cytokines, *e.g.*, IFNγ, IL-2, or of proliferative genes, respectively (Nika et al. 2010; Roifman and Grunebaum 2013). IL-2 represents a key mediator in the activation and proliferation of T-cells, and binds to receptor IL-2R, which not only has a crucial role in T-cell activation, but also promotes the progression of self-tolerance and regulates the functionality of natural killer (NK) cells (Nika et al. 2010; Roifman and Grunebaum 2013).

The effect of VacA on T-cell processing was investigated by the use of Jurkat cells, a transformed human T-cell line. Genes associated with apoptosis, signal transduction, NF-κB-dependent signaling or inflammation, *e.g.* IL-8 and IL-2R, were found to be upregulated (Takeshima et al. 2009). The stimulatory effect of VacA on the local immune response of Jurkat cells was confirmed using isogenic *H. pylori* ΔvacA mutants (Takeshima et al. 2009). Although it is well known that IL-2 stimulates T-cell proliferation in Jurkat cells, inhibition of proliferation was found in human $CD4^+$ T-cells, but these different responses are neither dependent on IL-2 expression nor on NFAT activation (Sundrud et al. 2004). In addition, a role of the N-terminal hydrophobic domain of VacA in mediating signaling to human T-cells and Jurkat cells was demonstrated (Sundrud et al. 2004). Making use of the known blocking effect of NPPBs (non-specific chloride channel inhibitors) on VacA activity, it was shown that VacA might hamper T-cell activation by a channel-independent mechanism in Jurkat cells (Boncristiano et al. 2003). In this process, two regions in the VacA protein, named i1 and i2, seem to be crucial for cell type specificity; moreover, VacA was shown to inhibit the activation of NFAT in T-cells by preventing the nuclear translocation (Boncristiano et al. 2003; Gebert et al. 2003; González-Rivera et al. 2012). Further, VacA can bind to receptors being expressed on

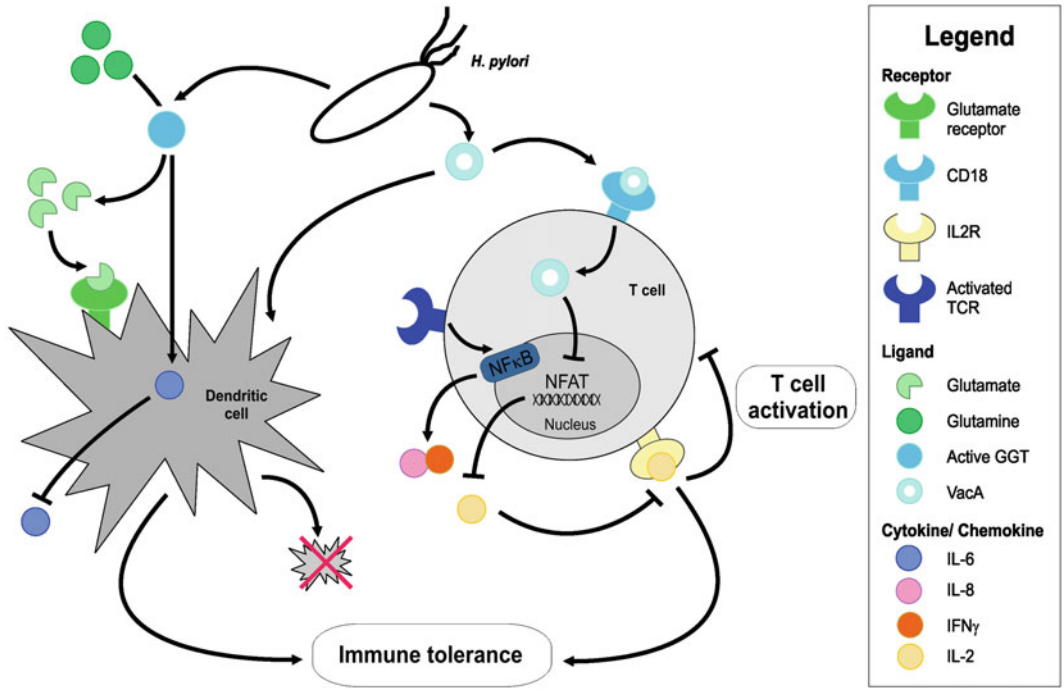

Fig. 1 *H. pylori* GGT- and VacA-mediated responses in immune cells of the host. GGT was found to activate the glutamate receptor on DCs and T-cells by increasing the glutamate concentration in the microenvironment through conversion of glutamine to glutamate. This interaction suppressed the secretion of pro-inflammatory cytokine IL-6 and induced tolerance in DCs. Moreover, GGT-primed tolerant DCs co-cultured with $CD4^+$ T-cells differentiated into Treg cells. It was also reported that GGT can manipulate T-cell proliferation through inducing cell cycle arrest. VacA binds to the CD18 receptor on T-cells for cellular entry and this inhibits the NFAT signaling and subsequent IL-2 production, which ultimately suppresses proliferation. Altogether, tolerated DCs, suppression of T-cells expansion and production of Tregs during interaction with *H. pylori* factors pave the way for immune tolerance and persistent *H. pylori* infection

T-cells beyond β-integrin, which results in the inhibition of IL-2 secretion and decreased NFAT activation (Sewald et al. 2008, 2011). Nevertheless, it was found that extracellular calcium ions are essential for the TCR-dependent NF-κB signaling. VacA is able to regulate the calcium ion influx, forcing NF-κB activation and thus elevating the pro-inflammatory response in human eosinophils (Kim et al. 2007a). Thus, *H. pylori* may manipulate the calcium balance during infection, which might be responsible for the VacA-dependent effect on NF-κB in T-cells (Liu et al. 2016). The role of VacA in NFAT- and IL-2R-dependent signaling is summarized in Fig. 1.

Interestingly, CagA acts as an antagonist of VacA with respect to NFAT activation (Yokoyama et al. 2005). Microarray-based analysis of CagA-transfected human gastric epithelial cells demonstrated that CagA activates NFAT signaling through induction of nuclear translocation of cytoplasmic NFAT (Yokoyama et al. 2005). For this, the EPIYA (Glu-Pro-Ile-Tyr-Ala)-containing region of CagA protein is essential, but CagA phosphorylation (which typically takes place during infection) is not required (Yokoyama et al. 2005).

4 Activities of VacA on Dendritic Cells and Macrophages

The apoptotic response of gastric epithelial cells and eosinophils upon exposure to VacA, as well as its immune regulatory effect on T-cells, have been well characterized (Calore et al. 2010; Kim

et al. 2010; Käbisch et al. 2016). However, VacA is also known to affect the maturation of DCs (Kim et al. 2011; Oertli et al. 2013). The manipulating effect of *H. pylori* VacA on DCs and the role in immune cell tolerance is schematically shown in Fig. 1. Immature DCs are located in peripheral tissue where they can be activated to undergo maturation by various antigens, including microbial peptides (Zanotti et al. 2002). Mature DCs can initiate an immune response by activating other immune cells. One of the earlier reports showed that expression of *cag*PAI or *vacA* genes was not required for the activation and maturation of DCs during *H. pylori* infection (Kranzer et al. 2005). However, in murine bone-marrow and splenic DCs derived from infected animals, the STAT3 (signal transducer and activator of transcription-3) pathway is activated (Kao et al. 2010; Oertli et al. 2012; Rizzuti et al. 2015). In this model, stimulation by *H. pylori* and its secreted virulence factors result in increased levels of the pro- and anti-inflammatory cytokines IL-1β and IL-10, respectively, comparable in strength to induction by *Escherichia coli* LPS (Kao et al. 2010; Oertli et al. 2012; Rizzuti et al. 2015). This suggests that these cytokines affect STAT3 regulation during *H. pylori* infection. Moreover, chemical or genetic inhibition of STAT3 led to an up-regulated DC maturation, indicating that STAT3 inhibits DC activation (Melillo et al. 2010). Since IL-10 promotes STAT3 activation, increasing the amount of secreted IL-10 would hamper the activation of DCs (Braun et al. 2013). These authors have shown that in addition to IL-10, IL-6 can activate the STAT3 pathway. Furthermore, it was shown that IL-6 mediated STAT3 activation leads to a transient pro-inflammatory response, while IL-10 based effects might continuously act anti-inflammatory (Braun et al. 2013). It can be concluded that a cytokine imbalance results from various *H. pylori* virulence factors that might alter activation or maturation of DCs.

Contrasting findings have been reported regarding the apoptotic effect of *H. pylori* virulence factors on DCs. In monocyte-derived DCs exposed to *H. pylori*, the induction of apoptosis was not detected (Galgani et al. 2004). However, analyzing the direct effect of VacA on human DCs, Kim et al. (2015a) identified that VacA can induce endoplasmic reticulum (ER) stress which can lead to apoptosis. Moreover, ER stress seems to occur earlier than the induction of apoptosis, so that ER stress might be the critical inducer for the regulation of apoptotic processes in DCs (Kim et al. 2015b). Regarding the development of tolerogenic DCs, *H. pylori* infection experiments performed *in vitro* and *in vivo* have demonstrated promoting effects, but the host cell mechanisms behind these observations remain currently unclear (Calore et al. 2010; Necchi et al. 2009).

Apart from affecting DC maturation, *H. pylori* virulence factors including VacA are further known to act on monocytes and macrophages during infection. As Allen (2007) already summarized, a variable ability of human monocytes or macrophages to kill *H. pylori* was experimentally shown. Compared to monocytes, macrophages exhibit a reduced capacity to kill *H. pylori* (Allen 2007; Borlace et al. 2008) for as yet unknown reasons. To investigate a possible protective effect by CagA or VacA against killing, primary human monocytes and macrophages were infected with strains of *H. pylori* differing in their CagA expression and VacA activity; however, after 48 h of infection no correlation was found between the number of viable bacteria and thus the cell's ability to kill *H. pylori*, and CagA expression or VacA activity (Borlace et al. 2008). The *cag*PAI plays no role in survival of the bacteria inside phagocytic cells (Odenbreit et al. 2001) and the *vacA* status of the bacteria is non-determinative for the phagosome fusion in human monocytes (Rittig et al. 2003). In contrast, other studies have indicated that VacA is crucial for intracellular survival and phagosome maturation (Petersen et al. 2001; Terebiznik et al. 2006). A link between urease activity and VacA for the survival in macrophages was indicated by Schwartz and Allen (2006). Infection of the monocytic cell line THP-1 by *H. pylori* for 8 h revealed an elevated amount of oncostatin M

(belonging to the group of IL-6 cytokines) in the supernatant, indicative of a pro-inflammatory response (Zeaiter et al. 2011). However, using isogenic deletion mutants it was shown that this pro-inflammatory response is independent of VacA, CagA or T4SS (Zeaiter et al. 2011). Thus, other virulence factors of *H. pylori* can be postulated to affect the pro- and anti-inflammatory cytokine secretion in monocytes and macrophages, but their nature remains to be investigated in future studies.

5 GGT Manipulates T Cell Proliferation and Cell Cycle Progression

Various studies described an *H. pylori*-associated inhibitory effect on cell growth leading to cell cycle arrest (Lew et al. 1991; Wagner et al. 1997; Chiou et al. 2003). Both CagA and VacA inhibit T-cell activation, while proliferation of these cells can also be inhibited by incubation with bacterial supernatants or purified VacA (Gebert et al. 2003; Sundrud et al. 2004). Moreover, as discussed above, infection with *H. pylori* leads to inhibition of T-cell proliferation by initiating apoptotic pathways (Wang et al. 2001). However, studies by Gebert and co-workers (2003) have shown that deletion of *vacA* did not hamper the effect on T-cell proliferation. In contrast, Sewald et al. (2008) have determined that VacA binds only to active human T-cells, leading to internalization of the virulence factor, whereas it does not bind to non-activated T-cells (Sewald et al. 2008). Moreover, this functional uniqueness of human T-cells compared to murine humanized T-cells indicates an important specificity of *H. pylori* VacA to host immune cells (Sewald et al. 2008). Since it was shown that during infection with *H. pylori* the proliferation of T-cells is inhibited by inducing cell cycle arrest, virulence factors other than CagA or VacA may be responsible for this phenotype (Gerhard et al. 2005).

Apart from its well-characterized importance for colonization *in vivo*, GGT has been shown to play a role in *H. pylori*-mediated apoptosis by mitochondrial pathways in epithelial cells (Kim et al. 2007b). In addition, it was demonstrated that *H. pylori* GGT is linked to blockage of T-cell proliferation and function, leading to immune evasion (Wüstner et al. 2015). It had previously been described that GGT is involved in proliferation of T-cells and in initiation of G1 arrest (Schmees et al. 2007). Using *H. pylori* isogenic Δ*ggt* deletion mutants, it was shown that the gene is crucial to suppress the proliferation of antigen-stimulated primary human T-cells and this effect could be replicated with recombinant *H. pylori* GGT, but not by mammalian GGT (Schmees et al. 2007). By incubating AGS gastric epithelial cells with recombinant *H. pylori* protein, GGT inhibition of cell cycle progression at G1 to S phase transition was demonstrated (Wüstner et al. 2015). These authors suggested that the arrest might depend on the growth characteristics of the target cells. Additionally, *H. pylori* seems to alter the expression of cell-cycle specific mediators, which would result in a dysregulated cell cycle progression during *H. pylori* infection (Wüstner et al. 2015). However, the exact mechanisms behind these processes require further investigation.

6 Function of the Neutrophil Activating Protein NapA

Recently, a correlation between the *H. pylori* virulence factor NapA and *H. pylori*-associated peptic ulcer disease was determined, although no correlation between other *H. pylori* associated diseases such as gastritis could be detected, neither for NapA nor for the virulence factors CagA, VacA, UreA, or UreB (Oktem-Okullu et al. 2015). It was already known that NapA is crucial for activation of neutrophils, which seems to promote damage of stomach tissue (Dundon et al. 2001). As recently reviewed elsewhere, NapA interacts with TLRs during infection (Amedei et al. 2006; Pachathundikandi et al. 2015). This TLR signaling induces neutrophil trans-endothelial migration and is involved in complex

host cell processes, and in the case of *H. pylori* this results in persistent colonization and chronicity of infection (Brisslert et al. 2005). Using purified recombinant *H. pylori* NapA or bacteria secreting the protein, these authors were able to show that treatment induces transendothelial transmigration of neutrophils in a CagA- and VacA-independent manner (Brisslert et al. 2005). The continuous recruitment of neutrophils to the site of infection had already been shown by others (Go 1997; Del Giudice et al. 2001). It is likely, however, that other chemotactic effector molecules, in addition to NapA, are involved in the migration of neutrophils (Satin et al. 2000; Brisslert et al. 2005). An additional role for NapA was shown in the T-cell dependent immune responses (Amedei et al. 2006). The effects of NapA on transmigration of neutrophils and the role in reactive oxygen species release are summarized in Fig. 2.

Previously, it was shown that iron can be stored in the cavity of NapA, although crystal structure analyses failed to detect iron (Tonello et al. 1999; Zanotti et al. 2002). The presence of high amounts of basic residues typically present in chemokines or cytokines might be crucial for the neutrophil activating function of NapA (Collaborative Computational Project, Number 4 1994; Yang et al. 1994; Zanotti et al. 2002). NapA and urease both seem to be important for the recruitment of neutrophils to the site of infection and may be involved in the response to oxidative stress (Wang et al. 2006). In a further study, expression of NapA and the outer membrane protein (Omp) 18, which is expressed in a limited number of *H. pylori* strains, correlated

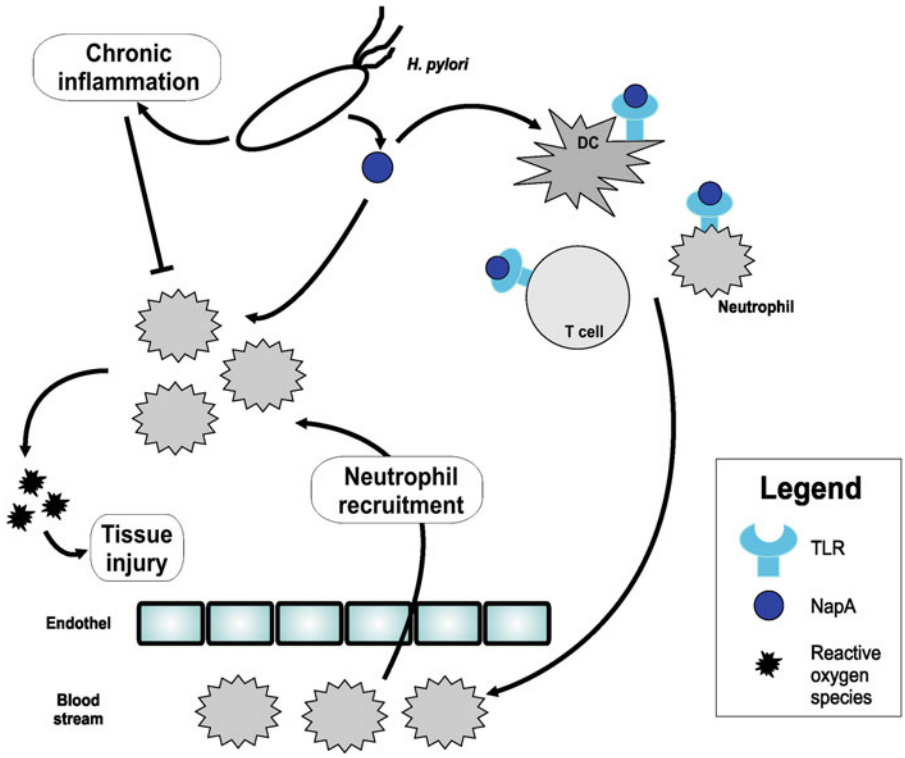

Fig. 2 Neutrophil immunity control during *H. pylori* infection. NapA of *H. pylori* is named for its role in the recruitment and activation of neutrophils during infection. NapA-induced activation of neutrophils leads to the production of reactive oxygen species and related tissue injury, which supports chronic inflammation. In addition, NapA can bind to TLR2 on neutrophils and monocytes to induce pro-inflammatory cytokine production and furthermore induces a pro-inflammatory Th1 phenotype. Therefore, NapA-mediated above effects ultimately result in epithelial barrier disruption and increased inflammatory reactions

with IFNγ-mediated immune response (Shan et al. 2015). During infection with a Δ*omp18* deletion strain, NapA expression was upregulated when the cells were co-incubated with IFNγ, suggesting NapA expression might be modulated by Omp18 in an IFNγ-dependent manner (Shan et al. 2015). However, the immune regulatory mechanisms behind the Omp18-dependent effect on NapA expression and neutrophil activation remained as yet unclear.

7 Role of Cholesterol in *H. pylori* Interactions with Immune Cells

Cholesterol is an important component of mammalian cellular membranes and has physiological roles in fat metabolism. In contrast, the membranes of many prokaryotes do not contain cholesterol, and most bacteria do not possess the genes required for cholesterol synthesis. *H. pylori* has evolved mechanisms to extract cholesterol from the host's cellular membranes and converts into glucosides by using a specific enzyme, CGT (Wunder et al. 2006). It was a remarkable finding that a common component of host membranes is utilized by a pathogen, to employ for virulence. Cholesterol acquisition appears to be essential for *H. pylori* survival in the human host and for prevention of effective host immune attacks (Wunder et al. 2006). *H. pylori* cells exhibit a high affinity for cholesterol and the chemotactic bacteria follow a cholesterol gradient, even responding to 20 times lower cholesterol concentration than are normally present in serum. It has been shown that the bacteria extract cholesterol from cell membranes when co-cultured with epithelial cell lines and convert to α-glucosides such as cholesteryl-α-glucoside, cholesterylacyl-α-glucoside or cholesteryl-phosphatidyl-α-glucoside by means of the CGT glucosyltransferase (encoded by gene HP0421, also known as *cgt* or *cap*J). This enzyme is necessary for prevention of phagocytosis by macrophages. However, when the bacteria were artificially loaded with high levels of cholesterol, this actually increased phagocytosis rates, indicating that only a high enough ratio of metabolized α-glucosides per cholesterol (unconverted) prevents engulfment. In addition, cholesterol glucosylation was also found to reduce the T-cell responses against *H. pylori* (Wunder et al. 2006). A recent study supported the role of cholesterol acquisition and its modification in *H. pylori* virulence. A murine macrophage cell line was used to show that phagocytosis of wild-type bacteria was delayed and phagosome maturation in infected cells was inhibited, but not when the cells were infected with a knock-out Δ*cgt/capJ* mutant. The interference in phagocytosis and phagosome trafficking was dependent on lipid raft formation and phosphoinositide-3-kinase (PI3K) signaling (Du et al. 2016).

It was further reported that CagA co-fractionates with redistributed MARK2/Par1b in the detergent-resistant membrane fraction of infected AGS cells (Zeaiter et al. 2008). In addition to CagA, VacA was reported to be co-localizing in the lipid raft regions during infection and cholesterol depletion disrupted this localization (Nakayama et al. 2006; Lai et al. 2008). The methyl-β-cyclodextrin mediated cholesterol depletion disrupted CagA translocation and phosphorylation in infected cells. CagA-induced cellular elongation and IL-8 secretion were also severely affected in this process. This shows that the capacity to bind and extract cholesterol acts as a point of delivery for virulence factors in the lipid raft regions of infected cells. However, the overall adherence of the bacteria to epithelial cells is not affected by cholesterol depletion; this can be explained by the presence of other cholesterol-independent adherence factors of *H. pylori* (Lai et al. 2008).

A study involving various N-terminal and C-terminal truncation mutants of CagA revealed that the EPIYA containing C-terminal domain may directly interact with cholesterol to induce IL-8 secretion (Lai et al. 2011). Mutation of the *capJ* gene prevented CagA phosphorylation, c-Src and FAK (focal adhesion kinase-1) dephosphorylation and the subsequent elongation of *H. pylori* infected AGS cells. By complementation with CapJ or supply of exogenous CGs (cholesterol glucosides), the CagA function in the *capJ* mutant was restored. Infection with wild-

type *H. pylori* showed that lipid raft components were recruited to the site of attachment and exogenously supplied fluorescence tagged cholesterol/CGs co-localized in this area (Wang et al. 2012).

The cholesterol acquisition and its subsequent conversion were thought to be independent processes, but the discovery of the flotillin-like protein (HP0248) in detergent resistant membrane fractions of *H. pylori* changed that view. HP0248 was found to be important for cholesterol sequestration, as infection with a Δ*HP0248* mutant severely affected cholesterol accumulation in the bacterial membrane (Hutton et al. 2017). This mutation also affected CagA translocation, cell scattering and IL-8 secretion, which indicates the protein has an important role in virulence, and this was confirmed using a mice model of infection (Hutton et al. 2017). Moreover, it was shown that the T4SS pilus protein CagL interacts with the host cell receptor $\alpha_5\beta_1$ integrin during CagA delivery (Kwok et al. 2007; Conradi et al. 2012; Barden et al. 2013). This $\alpha_5\beta_1$ integrin co-localizes with cholesterol-rich micro-domains within the membrane, which supports the concept of CagA delivery in a cholesterol-dependent manner. In addition, $\alpha_5\beta_1$ integrin in cholesterol-rich micro-domains is required for the delivery of peptidoglycan to NOD1 (nucleotide binding oligomerization domain containing 1) recognition and NF-κB activation. The depletion of cholesterol from cell surfaces with methyl-β-cyclodextrin resulted in reduced NF-κB activation and IL-8 secretion in *H. pylori* infected epithelial cells (Hutton et al. 2010).

H. pylori growth in the presence of cholesterol substantially increased its resistance to antimicrobials, although phosphorylation of lipid-A played a major role in this process (McGee et al. 2011). A recent study discovered another important aspect of cholesterol glucosylation in the immune response against *H. pylori*. Wild-type but not Δ*cgt/capJ* bacteria blocked IFNγ signaling through decreased phosphorylation of Janus kinase (JAK) and STAT1 in infected primary gastric cells and gastric epithelial cell lines (Morey et al. 2018). The disruption of IFNγ signaling was due to the destruction of lipid rafts (mediated by cholesterol depletion), while cholesterol coating of infected cells regained the signaling activation. It was found that *H. pylori* infection disrupted the distribution of receptors IFNGR1 and IFNGR2 in lipid rafts, which provides an explanation for these observations. Lipid raft disruption also inhibited the responses to IFNβ, IL-6 and IL-22 and subsequent signaling (Morey et al. 2018). Cytokine-induced hBD3 (human-β-defensin 3) expression was also downregulated during *H. pylori* infection. Infection of cells with wild-type or Δ*cgt/capJ H. pylori* induced almost identical changes in gene expression. However, cholesterol depletion by wild-type bacteria suppressed the immune responses in infected cells, while non-infected cells (thereby not suffering from cholesterol depletion) in the vicinity may get inflamed by increased cell signaling, induced by bacterial factors that were released in the microenvironment (Morey et al. 2018). The above data show that cholesterol acquisition from host cell membrane helps *H. pylori* to deliver virulence factors, interferes with phagocytosis and also contributes in the manipulation of important host cell signaling mechanisms for the benefit of survival and persistence.

8 *H. pylori* Manipulates Antigen Presentation and Bacterial Recognition

Infecting bacteria are normally subject to phagocytosis, but many reports have shown that *H. pylori* interferes with this defensive process. In one of the earlier reports, Ramarao and co-workers (2000) showed that *H. pylori* inhibits the phagocytic function of neutrophils and monocytes, an inhibition that was dependent on proteins VirB7 and VirB11 of the T4SS. It was also documented that phagocytosis of *H. pylori* type-I strains by macrophages got delayed, whereas type-II strains were easily phagocytosed (Allen 2007). In addition, *H. pylori* prevented phagosome maturation and instead resulted in formation of a hybrid phagosome-endosome-lysosome with no or strongly reduced degradation (Borlace et al. 2011). This is a clear indication for

reduced antigen epitope production and MHCII presentation and provides an example of a successful bacterial adaptive response at the most apt time to avoid infection clearance. *H. pylori* VacA can inhibit CD74 (Ii)-dependent MHCII antigen presentation, but not the independent pathway of recycling MHCII presentation (Molinari et al. 1998). Moreover, CD74 is up-regulated on the surface of gastric cells during *H. pylori* infection and was reported to act as a receptor for *H. pylori* urease. CD74 is well known for its role in antigen presentation as it directs MHCII molecules to the endosomes, where it is partly digested to relieve the cleft of the MHCII molecule for peptide loading. It is plausible that CD74 binding to *H. pylori* urease interferes with MHCII localization and antigen loading for activation of adaptive immunity (Beswick et al. 2005; Beswick and Reyes 2009).

At least in mice, the important role of TLRs in the development of adaptive immunity against *H. pylori* infection is evident from several reports (reviewed by Pachathundikandi et al. 2015, summarized in Fig. 3). One of the major findings was the TLR-mediated MyD88 (myeloid differentiation-88) signaling on antigen presentation and co-stimulation in infected DCs for development of an adaptive immune response against *H. pylori* infection (Rad et al. 2007). In contrast, it was also found that at the initial phase of infection *H. pylori* can reside and replicate in macrophages, DCs or epithelial cells inside double-membrane auto-phagosomes, which later fuse with lysosomes and are almost fully degraded at 48 h of infection (Wang et al. 2009, 2010). *H. pylori* infection reduced the cell surface expression of MHCII, CD80 and CD86 instead these molecules were co-localized with the *H. pylori* containing vacuoles, which supports the interference on antigen loading and trafficking to the cell surface. Moreover, formalin-fixed bacteria exhibited the same effect. However, in infected DCs, MHCI expression was enhanced (Wang et al. 2010). These authors found that TLR2 and TLR4 are necessary for the interference on MHCII trafficking and subsequent antigen presentation, which suggests an important role of *H. pylori* LPS in this mechanism (Wang et al. 2010).

Recently, it was also reported that miR-30b was upregulated in patients with chronic *H. pylori* infection and was targeting autophagy proteins ATG12 (autophagy related protein 12) and BECN1 (Beclin 1). The control of autophagy in *H. pylori* infected cells resulted in increased intracellular survival and bacterial replication (Tang et al. 2012). This shows a different-level control on autophagy and intracellular growth of *H. pylori,* which ultimately influences the antigen presentation. Furthermore, CD300E was identified as a new factor involved in *H. pylori* mediated interference on antigen presentation (Pagliari et al. 2017). Differentiation of monocytes to macrophages normally reduces the expression of CD300E, however, *H. pylori* infection increased the expression of this protein by down-regulating miR-4270, a miRNA that targets *CD300E* mRNA. This increased expression and activation of CD300E by *H. pylori* alone and agonistic antibody treatment drastically reduced the expression of MHCII molecules, thereby reducing antigen presentation and that prevented activation of T-cells (Pagliari et al. 2017). The above studies clearly affirm the interference on antigen presentation in *H. pylori* infection.

The interference on antigen presentation could be responsible for both the reduced recognition of antigens by T-cells and the ineffective adaptive immune response against *H. pylori*. DC maturation is an important step in the transition from antigen-capturing to antigen presentation, which is necessary for T-cell priming and activation. VacA suppresses DC maturation by downregulating the surface expression of MHCII, CD40, CD80 and CD86, apart from reducing migratory power of DCs (Molinari et al. 1998). The increased expression of PD1-L1 (Programmed cell death protein ligand-1)/B7-H1 in gastric epithelial cells was reported in *H. pylori* infection, which is independent of the virulence factors CagA, VacA or urease, but is enhanced by IFNγ (Das et al. 2006). In addition, gastric epithelial cells are expressing MHCII and co-stimulatory molecules such as CD80 and CD86, and this antigen presenting capacity

helps to activate naïve T-cells at the epithelial contact site. This presumably leads to clonal differentiation to enable well-developed adaptive responses against bacterial infection. However, *H. pylori* induced expression of PD1-L1 and the binding of this to PD1 on T-cells led to the suppression of antigen-specific T-cells, while it supported the maintenance of $FoxP3^+$ Treg cells (Beswick et al. 2007; Zhang et al. 2016). Induced expression of PD1-L1 in gastric epithelial cells along with other factors appears to maintain the Treg pool reported in gastric mucosa of *H. pylori* infected individuals (Cook et al. 2014; Hussain et al. 2016).

9 TLR Signaling in Immune Cells Induced by *H. pylori*

TLRs constitute a group of host cell surface and subcellular transmembrane proteins, which detect intruding microbes or microbial products outside the cells through their varied presence on the cell surface (TLR1, TLR2, TLR4, TLR5, and TLR10) or in intracellular compartments (TLR3, TLR7, TLR8, and TLR9) (Beutler 2009). These type I transmembrane glycosylated protein receptors are germ line encoded proteins composed of an ectodomain containing leucine-rich repeats, a transmembrane region, and an intracytoplasmic Toll/IL-1 receptor (TIR) domain. TLRs sense the presence of various PAMP or MAMP (microbe associated molecular patterns) and can thus detect bacteria, viruses, or fungi, which leads to the induction of downstream signals. TLR signaling can be divided into MyD88 dependent and TRIF (TIR domain containing adaptor inducing interferon-β) dependent signaling, both of which ultimately leads to activation of NF-κB, AP-1 and interferon regulatory factors (IRFs) for the production of chemokines, cytokines and type I interferons that activate the host immune system to control an infection (Pachathundikandi et al. 2011, 2015).

Early studies reported the difference on *H. pylori* LPS activation of NF-κB through TLR4 in epithelial cells and monocytes (Ishihara et al. 2004). In uninfected individuals, TLR4 expression was noticed only in lamina propria mononuclear cells, but *H. pylori* infection induced the expression of TLR4 and MD2 in gastric epithelial cells as well. Gastric epithelial cell lines expressing TLR4 and MD2 did not activate NF-κB when treated with *H. pylori* LPS, whereas THP1 cells responded with a robust activation (Ishihara et al. 2004). TLR4 and CD14 were involved in the activation of NF-κB in infected THP1 monocytes but not in the MKN45 gastric epithelial cell line. Mouse macrophages with a point mutation in their TLR4 gene showed decreased activation of NF-κB and TNFα (tumor necrosis factor-α) secretion upon *H. pylori* infection compared to wild type macrophages. Moreover, incubating monocytes with *H. pylori* culture supernatant resulted in NF-κB activation, but bacterial contact or a functional *cag*PAI was necessary for activation of epithelial cells (Maeda et al. 2001). Our studies showed that infection of THP1 cells with *H. pylori* increased the expression of TLR2 and TLR5 as well as secretion of IL-8 and TNFα in a *cag*PAI dependent manner (Pachathundikandi et al. 2011). In contrast, IL-8 production in mouse macrophages was mediated through TLR4 in response to *H. pylori* LPS, whereas intact bacterial cells of *H. pylori, Helicobacter hepaticus* or *Helicobacter felis* resulted in a response mediated through TLR2. These bacterial infections also induced IL-6 expression in a TLR2 dependent manner (Mandell et al. 2004).

Apart from LPS, at least three *H. pylori* proteins have been identified that can activate TLR2. *H. pylori* Hsp60 (also called GroEL) was shown to induce IL-8 secretion mediated by MAPK signaling in a human monocytic cell line (Zhao et al. 2007). NapA induced the production of IL-12 and IL-23 in primary neutrophils and monocytes and induced a Th1 response in T-cell clones prepared from healthy donors, while it shifted the Th2 response to Th1 in cells obtained from allergic donors (Amedei et al. 2006). Moreover, T-cell clones prepared from gastric mucosa of infected patients were found to be of the cytotoxic Th1 phenotype and produced TNFα (Amedei et al. 2006). Further, *H. pylori* UreB could activate TLR2, resulting in an increased

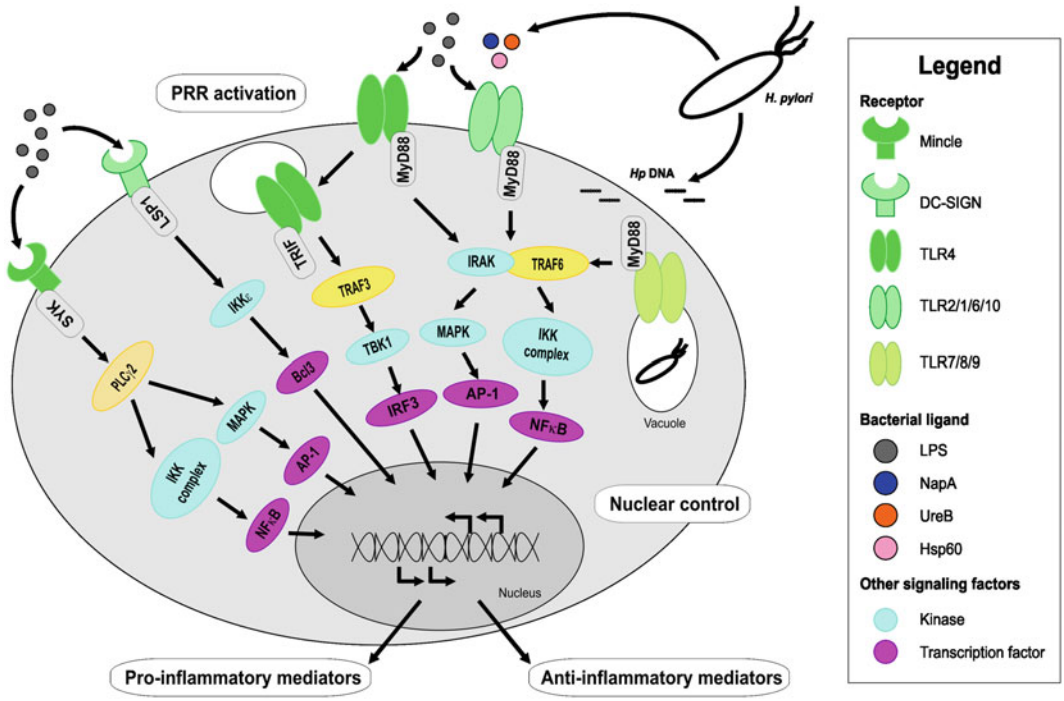

Fig. 3 The pro- and anti-inflammatory signaling through different PRRs during *H. pylori* infection. *H. pylori* is reported to interact with various PRRs such as TLRs and DC-SIGN during infection with host cells. This ultimately leads to the induction of various signaling pathways to activate or modify functions of transcription factors such as NF-κB, AP-1 and IRFs. These multiple induction and modification mechanisms resulted in the production of pro- and anti-inflammation states in *H. pylori* infection. Thus, different signaling mediated variation in immune responses may ultimately determine the outcome of associated diseases

expression of NLRP3 (NOD-like receptor pyrin domain-containing-3) and inflammasome assembly, while Δ*ureB* mutant bacteria inhibited caspase-1 activation in murine and human DCs (Koch et al. 2015). Thus roles for HSP60, NapA and UreB in TLR2 activation have all been demonstrated (summarized in Figs. 3 and 4).

H. pylori infection of mouse DCs induced the expression of IL-12 and IL-10 through TLR4/MyD88 signaling, whereas secretion of IFNα was increased substantially in *myd88* deficient cells. Moreover, IL-6 and IL-1β expression was decreased in *tlr2* deficient cells infected with *H. pylori* (Obonyo et al. 2007). We found that HEK293 cells stably expressing TLR2 (HEK293-TLR2) differentially expressed *IL-1β* but not *IL-6* during infection with *H. pylori*, however, *TNFα* expression was induced in both HEK293-TLR2 and HEK293-TLR10 cells after infection (Pachathundikandi and Backert 2016). *H. pylori* LPS activated HEK293 cells jointly overexpressing TLR2 and TLR10 to induce NF-κB signaling for *IL-8* and *TNFα* expression (Nagashima et al. 2015). We have also shown highly induced expression of TLR10 in THP1 monocytes infected with *H. pylori* (Pachathundikandi and Backert 2016). It was reported that induction of pro-IL-1β expression in DCs by *H. pylori* depends on TLR2 and NOD1, while infected *tlr2* deficient cells suppressed pro-IL-1β expression more than *nod1* deficient cells (Kim et al. 2013). Moreover, this work demonstrated a cumulative effect in *tlr2-nod1* double deficient cells, which suggests that these two receptors have redundant roles in pro-IL-1β expression during *H. pylori* infection (Kim et al. 2013).

Mice deficient in *myd88* produced decreased gastric inflammation in response to *H. pylori* infection, but the gastric colonization levels

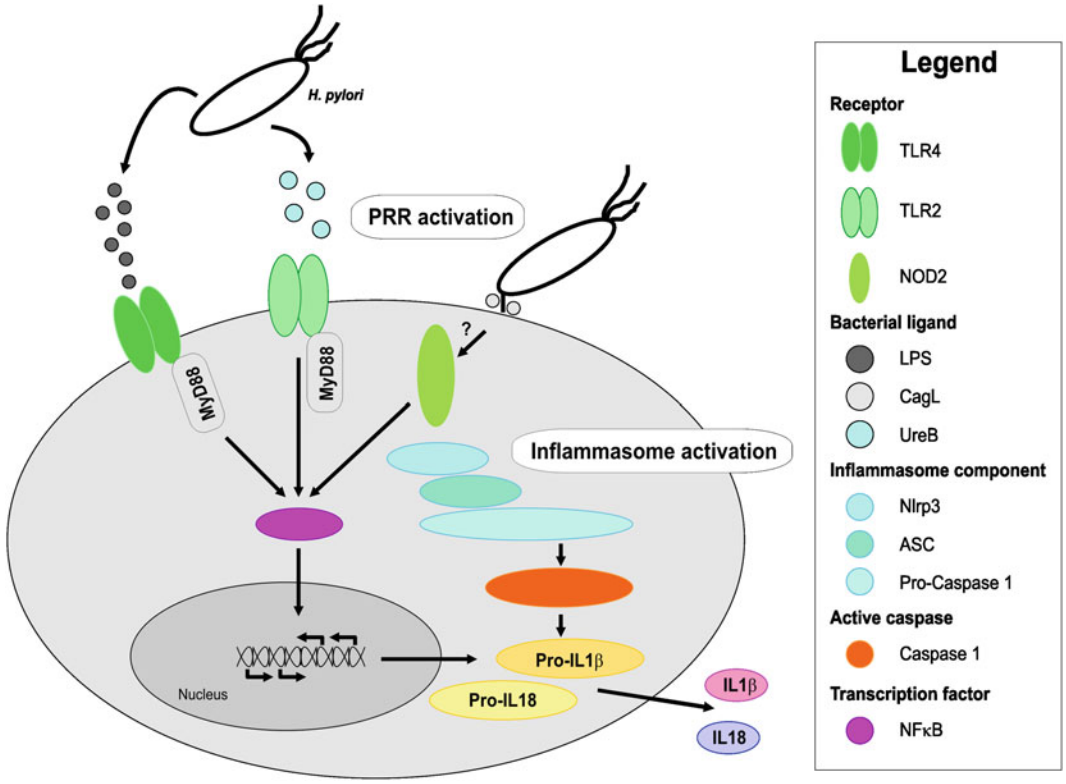

Fig. 4 TLR2- and NOD2-mediated inflammasome activation during *H. pylori* infection. It was found that *H. pylori* UreB activated TLR2 signaling for NLRP3 expression and assembly of the inflammasome in mice. However, pro-IL1β expression was induced by LPS through TLR4 signaling. The inflammasome induced activation of caspase-1 resulting in the production of active IL-1β and IL-18 and exerted various effects described in the text

were increased (Rad et al. 2007). The secretion of pro-inflammatory IL-6 and IL-12p40 cytokines by murine DCs infected with *H. pylori* lysates was largely dependent on TLR2 activation, whereas infection with live bacteria induced anti-inflammatory IL-10 secretion in a TLR2-dependent manner. *H. pylori* DNA or RNA was able to induce strong cytokine secretion in wildtype and *tlr2/4* deficient DC cells, an effect that was completely abrogated in *tlr2/4/9* deficient cells. However, *tlr9* deficient cells showed equivalent amounts of cytokines secretion when compared to wild type cells upon exposure to bacterial DNA or RNA (Rad et al. 2007). *H. pylori* RNA detection by DCs was dependent on TLR8 alone or possibly in combination with TLR7. In addition, exposure of DCs to *H. pylori* RNA resulted in type-I IFN production that was independent of TRIF or MyD88 but dependent on RIG1 activation (Rad et al. 2009).

The production of Treg cells ($CD25^{+}Foxp3^{+}$) in response to *H. pylori* presence is also proposed to be mediated through TLR involvement (Rad et al. 2006). TLR2-mediated activation of B-cells in mice infected with *H. felis* prevented immunopathology and pre-neoplastic changes through the production of a Treg cell population (Sayi et al. 2011). Instead, the adoptive transfer of *H. felis*-specific effector T-cells aggravated the immunopathology to produce pre-neoplastic changes and reduced colonization in infected *tcrβ/rag1* knockout, immune-deficient mice. However, co-transfer of effector T-cells and Treg cells to these infected mice exhibited alleviation of

symptoms in *tcrβ* deficient mice, but not in *rag1* deficient mice, which suggests B-cells are involved in this process. *H. felis* induced the activation of TLR2 in B-cells which led to an increased expression of co-stimulatory molecules CD40, CD80, CD86 and secretion of IL-10, IL-6, TNFα, and antibodies (Sayi et al. 2011). When murine DCs deficient in *tlr2* were infected with *H. pylori*, the cells produced more IFNγ and less IL-17 and IL-10 compared to wild type DCs. In addition, *tlr2* deficient mice expressed more IFNγ *in vivo* and less FoxP3, IL-10 and IL-17A in their infected gastric mucosa compared to infected wild type animals. These observations may explain the increased gastritis and lower colonization of bacteria in infected *tlr2* deficient mice. TLR2 mediated signaling during *H. pylori* infection produced tolerogenic DCs that dampened the immuno-pathological Th1 response and allowed higher colonization levels to be reached (Sun et al. 2013).

H. pylori infection increased the IRAK-M expression in *tlr2* deficient DCs. Infection of these DCs induced a more pronounced pro-inflammatory response through higher expression of MHCII, TNFα and MIP2 and reduced IL-10 expression, although Treg and Th17 responses were comparable to those seen in wild-type mice (Shiu et al. 2013). TLR9 expression in the gastric tissue was increased after *H. pylori* infection in mice and this elevated expression was mostly observed in macrophages, DCs and $CD3^+$ cells. The *tlr9* deficient mice showed increased myeloperoxidase (MPO), TNFα and IFNγ expression during initial phase of *H. pylori* infection, but colonization levels were similar in wild type and deficient mice. The treatment of exogenous recombinant IFNα reduced the pro-inflammatory changes in the infected *tlr9* deficient mice (Otani et al. 2012).

The reports summarized here demonstrate that TLR activation plays a crucial role in innate and adaptive immune responses against *H. pylori*. The above data show that immune cell signaling in *H. pylori* infection is mainly carried out through TLR2, TLR4 and TLR9 receptors, with minor roles for TLR7, TLR8 and TLR10 (summarized in Figs. 3 and 4). These receptors are expressed at epithelial and immune cells alike, which are the two major cell types *H. pylori* interacts in the gastric mucosa. The most striking feature of this interaction is the dual role of TLR2 on activating both pro-inflammatory and anti-inflammatory responses, which may partly explain the varied disease outcome of infected individuals.

10 Pro- and Anti-inflammatory Signaling by *H. pylori*

The interaction between bacteria and their host leads to the activation of various pro- and anti-inflammatory signal transduction pathways from various PRRs, resulting in the activation of number of transcription factors in the host cells (Backert and Naumann 2010; White et al. 2015). Gastric epithelial cells, interacting with *H. pylori,* respond with various signals to initiate an inflammatory process in an attempt to control the infection. Several studies reported the involvement of different pathways in this process (summarized in Fig. 3). *H. pylori* T4SS has been shown to function as the conduit for entry of different factors such as effector protein CagA, heptose 1,7-bisphosphate (HBP), ADP-heptose, peptidoglycan as well as bacterial DNA, which ultimately activate these pathways (Backert et al. 2000; Viala et al. 2004; Varga et al. 2016; Gall et al. 2017; Pfannkuch et al. 2018). Some earlier studies ruled out the involvement of CagA in the NF-κB pathway, although it was reported that CagA can potentiate the NF-κB response through a protein interaction cascade of Grb2 → Ras→Raf → Mek → Erk for IL-8 secretion (Brandt et al. 2005). The transgenic CagA expression in mice, induced the PAR1 mediated IκB (Inhibitor kappa B) sequestering to lower the NF-κB threshold for activation (Suzuki et al. 2015). It was experimentally shown that outer membrane vesicles (OMVs) from *H. pylori* could deliver peptidoglycan to the cytosol of exposed cells, resulting in NOD1 mediated NF-κB activation and IL-8 and CXCL2 production (Kaparakis et al. 2010). Moreover, *H. pylori*

induced NOD1-dependent IFNβ secretion in turn activated the expression of IFN-stimulated gene factor 3 (ISGF3) and CXCL10; which protected the mice from infection (Kaparakis et al. 2010).

Activation of NOD1 by *H. pylori* resulted in responses that were augmented by IFNγ to produce various chemokines such as IL-8, CXCL10, CCL2, CCL3, CCL4, and CCL5. *H. pylori* infection further activated phosphorylation of IFNγ signaling factor STAT1 and expression of IRF1 via a NOD1-dependent mechanism. Furthermore, infection activated the production of Th1 cells and elevated the secretion of high amounts of IFNγ, which amplified the NOD1 response in a feedback manner. This synergistic action of NOD1 and IFNγ exacerbated the immune responses in the gastric mucosa during *H. pylori* infection; an observation in line with the finding that gastric cancer patients display upregulated expression of NOD1, IRF1 and IL-8 (Allison et al. 2013). In contrast, NOD1 activation by *H. pylori* suppressed the transcription factor CDX2 (caudal homeobox 2) in both normal and cancerous gastric epithelial cells; this factor is involved in intestinal metaplasia (Asano et al. 2016). Bile duct epithelial cells responded to *H. pylori* through NOD1 and MyD88 pathways to produce activation of NF-κB and IL-8 secretion, which was inhibited by pre-treatment with antibodies against $\alpha_5\beta_1$ integrin (Boonyanugomol et al. 2013). Trans-epithelial neutrophil migration was reported to be dependent on NOD1 mediated IL-8 secretion in *H. pylori* infection (Kim et al. 2015a). Finally, a recent study reported that $\alpha_5\beta_1$ integrin- and Src-mediated JNK/ERK signaling for NF-κB and AP-1 activation following *H. pylori* infection was independent of NOD1 and CagA, but required active CagL (Gorrell et al. 2013).

HBP is a bacterial metabolic intermediate of LPS biosynthesis. It was proposed that HBP can enter the cytosol of infected epithelial cells via the T4SS, and activates a novel signaling cascade involving alpha-kinase 1 (ALPK1) and the phosphorylation-dependent oligomerization of the TNF-α receptor-associated factor (TRAF)-interacting protein with forkhead-associated domain (TIFA) for NF-κB activation. TIFA deficiency or HBP mutants of *H. pylori* almost completely abrogated NF-κB mediated IL-8 production in infected epithelial cells. The major difference between *H. pylori* activated ALPK1-mediated TIFA signaling platform from that observed with other bacteria is the presence of TRAF2 instead of TRAF6 for the NF-κB activation (Stein et al. 2017; Gall et al. 2017; Zimmermann et al. 2017). However, very recent studies indicate that the translocated metabolite activating NF-κB may not be HBP, but ADP-heptose, produced downstream by the same LPS biosynthesis pathway (Zhou et al. 2018; Pfannkuch et al. 2018).

H. pylori infection can also activate EGFR (epidermal growth factor receptor) signaling for COX2 (cyclooxygenase 2) expression and PGE2 (prostaglandin E2) secretion in a *cag*PAI-dependent manner. *Egfr* deficiency reduced this COX2 induction and PGE2 expression in infected murine cells. Likewise, *H. pylori*-induced COX2 expression increased the survival of epithelial cell lines; this would suggest the existence of an EGFR mediated pro-cancerous signaling axis (Sierra et al. 2013). In addition, EGFR signaling is also involved in the pro-inflammatory reaction and epithelial DNA damage in *H. pylori* infection (Sierra et al. 2018). Furthermore, non-phosphorylated CagA activated two other transcription factors such as SRF (serum response factor) and ELK1 (ETS domain containing protein) (Hirata et al. 2002). ELK1 and SRF activation induced the intestinal cell specific marker Villin expression in *H. pylori* infected gastric epithelial cells (Rieder et al. 2005). Apart from that, *H. pylori* infection of gastric cancer cell lines increased the expression of SIAH2 (seven in absentia homologue 2) through the ETS2 (E26 oncogene homolog 2) and TWIST1 (twist related protein 1) transcription factors. The stable expression of SIAH2 increased the invasiveness and migration capacity of gastric cancer cells (Das et al. 2016).

H. pylori can also induce host anti-inflammatory responses, whose role is to dampen the ability to clear an infection, which is somehow essential for general inflammation control by the pathogen. The binding of *H. pylori* LPS to

DC-SIGN, a c-type lectin receptor (CLR), resulted in the signaling which blocked Th1 cell development and decreased production of IL-12 and IL-6 (Bergman et al. 2004; Gringhuis et al. 2009). DC-SIGN signaling can revert the TLR mediated pro-inflammatory cytokine expression to favor an anti-inflammatory cytokine IL-10 production through Raf1, however Raf1 deficiency did not suppress IL-10 expression in *H. pylori* infection (Gringhuis et al. 2007, 2009). When human DCs (derived from monocytes) were infected with *H. pylori,* it produced IL-10 in a DC-SIGN, TLR2 and TLR4 dependent manner, and interestingly all these receptors were reported to bind bacterial LPS (Chang et al. 2012). It was observed that, p38 signaling mediated NF-κB activation led to histone modification for upregulated expression of IL-10 in *H. pylori* infection (Chang et al. 2012). However, it was observed that IL-10 and CD40 expression reduced in *H. pylori* infected DCs obtained from gastric cancer patients, while infected T-cells exhibited decreased IL-17 expression (Chang et al. 2012). Recently, it was found that *H. pylori* can induce Th2 response through DC-SIGN mediated activation of an atypical NF-κB pathway. DC-SIGN interaction with fucose-containing moieties from *H. pylori* and *Schistosoma mansoni* induced the phosphorylation of LSP1 (lymphocyte specific protein 1) for IKKε activation to effect nuclear translocation of Bcl3 (B-cell lymphoma encoded protein 3) and association with p50 NF-κB subunit. This pathway resulted in the downregulation of pro-inflammatory cytokine production and upregulation of anti-inflammatory cytokine IL-10 and chemokines for specific recruitment of Th2 cells (Gringhuis et al. 2014).

Anti-inflammatory responses through CLR during *H. pylori* infection are not limited to DC-SIGN signaling, as MINCLE (macrophage inducible C-type lectin) is reported to induce IL-10 production in infected human macrophages; it was found that LPS can function as the ligand for this induction (Devi et al. 2015). The induced expression of small RNA miR-223–3p as well as secreted IL-10 in infected human immune cells were found to significantly downregulate the expression of inflammasome forming NLRP3, and this can interfere with the production of pro-inflammatory active IL-1β (Pachathundikandi and Backert 2018). The IL-25 (IL-17E) signaling induced Th2 response significantly reduced the inflammation in mice during infection with *H. pylori,* while IL-23 signaling increased this inflammation (Horvath Jr et al. 2012, 2013). Moreover, it was reported that *H. pylori* could induce HO-1 (heme oxygensase 1) expression through phospho-CagA dependent p38 signaling and nulcear factor E2-related factor 2 (NRF-2) activation. The HO-1 expression was found to be upregulated in tissue from infected patients as well as from infected mice (Gobert et al. 2014). Furthermore, deficiency of *hmox1* exacerbated the inflammation through increased M1 macrophage, Th1 and Th17 responses, which resulted in reduced *H. pylori* colonization (Gobert et al. 2014). Similarly, deficiency in *trpm*2 (transient receptor potential cation channel subfamily M member 2), a calcium channel protein, increased gastric inflammation and reduced the bacterial colonization in infected mice (Beceiro et al. 2017). TRPM2 deficient macrophages had altered calcium levels and produced more inflammatory mediators like IL-12, IL-6, IL-1β and TNFα. The oxidative stress was also increased, which attributed to the high expression of NADPH oxidase and iNOS due to enhanced MAPK signaling in TRPM2 deficiency. In addition, TRMP2 deficiency polarized the macrophages to a more pro-inflammatory M1 phenotype (Beceiro et al. 2017). Transgenic mice lacking Nogin, the BMP (bone morphogenetic protein) signaling inhibitor, showed exacerbated expression of pro-inflammatory cytokines during *H. pylori* infection, which suggests for an anti-inflammatory role of BMP in this infection (Takabayashi et al. 2014). *H. pylori* arginase (encoded by *roc*F) was found to suppress the expression of NF-κB family transcription factors and their corresponding cytokine genes. Infection with Δ*rocF* mutants induced more IL-8 in epithelial cells than wild type bacteria, which confirms a role for immunosuppression during infection (Kim et al. 2012). The gastric tissue TGFβ and

its receptor levels were both decreased in patients infected with *H. pylori,* which is known to have anti-inflammatory activities through suppression of T-cells, B-cells, macrophages and NK cells (Shih et al. 2005; Jo et al. 2010). Furthermore, the model proposed by Li and co-workers (2015) incorporated decreased TGFβ and receptors in acute *H. pylori* infection, while chronic infection increased their signaling activity. In conclusion, the above described pro- and anti-inflammatory signaling mechanisms and their timely or untimely induction would by and large determine the outcome of *H. pylori* infection.

11 Inflammasome Activation Through TLR2 and NOD2 Signal Transduction

The formation of the inflammasome is an important aspect of the innate immune response, constituting multiprotein scaffolds for the recruitment of zymogen pro-Caspase 1 to become activated through proximity induced autoproteolysis and subsequent production of active IL-1β and IL-18. Proteins belonging to the family of Nod-like receptors (NLRs) are the major contributors in this process. In particular, NLR pyrin domain containing 1 (NLRP1), NLRP3 and NLR card domain containing 4 (NLRC4) are the three major and best studied inflammasome forming proteins. The inflammasome can also form by activity of non-NLR factors; AIM2 (absent in melanoma 2) is the major non-NLR inflammasome forming factor for active IL-1β and IL-18 production. Other NLR proteins have also been implicated for the formation of the inflammasome, however, the mechanistic details of these are yet to be discovered (Backert 2016).

As could be expected, *H. pylori* infection activates the formation of the inflammasome both in mouse and human cells (summarized in Fig. 4). The production of active IL-1β and IL-18 have multiple roles in the fine tuning of the host's immune responses against the bacteria. Apart from its known role as a pyrogen, IL-1β can recruit and activate different leukocytes and result in production of multiple mediators of inflammation. IL-18 is involved in the modulation of T-cell responses, which depends on the presence of other mediators of inflammation. For instance, IL-18 can turn the fate of T-helper cells into either Th1 or Th2 phenotype and counter the pro-inflammatory response induced by IL-1β (Dinarello 2009). The activation of an inflammasome requires the optimal expression of crucial components such as NLRs, pro-Caspase 1, IL-1β and IL-18. Generally, TLR signaling activation of NF-κB induces the expression of these proteins. In case of *H. pylori* infection, TLR2 signaling was reported to be the rate-limiting step in the activation of NLRP3 inflammasome (Koch et al. 2015). A study using a transposon mutant library of *H. pylori* revealed that urease deficient bacteria were no longer able of such activation in DCs (Koch et al. 2015). Infection of DCs with specific Δ*ure*A and Δ*ure*B mutants pinpointed the role of UreB in this activation (Koch et al. 2015). However, pro-IL-1β expression was induced through *H. pylori* LPS mediated activation of TLR4, as particular LPS mutants failed to do so. This shows the need for concerted action of TLRs in the activation of the inflammasome in *H. pylori* infection. The *tlr2* and *nlrp3* deficient mice showed an increase in IFNγ producing $CD4^+$ cells and reduced colonization of *H. pylori* (Koch et al. 2015). Mice deficient in either *casp1, il18,* or *il18r* genes also revealed lower *H. pylori* colonization rates. This shows that TLR2 mediated NLRP3 inflammasome activation in mice benefits *H. pylori* colonization and persistence. Wild-type mice infected with Δ*ure*B mutants had more IFNγ producing $CD4^+$ cells compared to animals infected with wild type bacteria; likewise, *tlr2* or *nlrp3* deficient mice produced lower levels of these cells when colonized with either type of bacteria (Koch et al. 2015). Moreover, it was reported that animals with allergic asthma had reduced pathologies when neonatal mice were infected with wild-type *H. pylori,* but absence of this effect in case of the Δ*ureB* mutant suggests that UreB-induced immunity can protect the host from allergic asthma (Koch et al. 2015). This observed protection was abrogated by treatment of blocking antibodies raised against IL-18.

Moreover, adoptive transfer of $CD25^+$ Treg cells from mice infected with wild type *H. pylori* alleviated the asthma pathologies (Koch et al. 2015).

Neither VacA nor CagA were essential for inflammasome activated IL-1β secretion, but a bacterial mutant lacking T4SS pilus protein CagL resulted in decreased activation, which hints to a role of intact T4SS in this process (Kim et al. 2013). In this study, expression of *il1β* and *nlrp3* was found to depend on TLR2 and NOD2. DCs from either *tlr2* or *nod2* deficient mice significantly reduced the activation of the inflammasome, while in double deficient mice this effect was augmented (Kim et al. 2013). DCs deficient of *casp1* expressed similar amounts of pro-IL-1β as wild type mice upon infection with the bacteria, but were no longer able to secrete active IL-1β (Kim et al. 2013). These murine infection experiments showed that *H. pylori* colonization was increased in *il1, il1r,* and *casp1* deficient mice compared to wild type mice, but *nlrp3* deficient mice resulted in normal colonization, which contrasts with the above-mentioned study (Kim et al. 2013). In conclusion, TLR2, along with NOD2, is involved in the activation of the NLRP3 inflammasome during *H. pylori* infection and this process requires bacterial factors such as UreB, LPS and CagL.

12 Resolution of Inflammation by *H. pylori*

Inflammation typically undergoes three phases: acute inflammation, onset of resolution, and finally resolution to regain homeostasis. The acute inflammatory stage is marked by infiltration of neutrophils and monocytes at the inflamed site, producing classically activated M1 macrophages and resulting in the production of more pro-inflammatory mediators. This results in long-term adaptive immunity against the invader and works as a memory system to prevent future attack. At the onset of resolution, the second stage is characterized by reduced secretion of pro-inflammatory cytokines and chemokines. Neutrophils start to produce microparticles and the acute-phase production of lipid pro-inflammatory mediators (e.g. prostaglandins) switches to production of pro-resolution lipid mediators such as lipoxins, resolvins, maresins and protectins. In addition, more anti-inflammatory cytokines such as IL-10 and TGFβ are produced during this stage and thereby induces more M2 macrophages, these cells are necessary for resolution to gradually restore homeostasis with assistance of other factors. (Ortega-Gómez et al. 2013; Sugimoto et al. 2016).

H. pylori infection is a chronic condition that in humans typically starts during childhood and lasts for a life time if not eradicated by antibiotic therapy. However, the majority of infected individuals do not develop associated complications like peptic ulcer, gastric cancer or MALT lymphoma, despite the fact that gastritis and local inflammation of the gastric mucosa probably appears in most infected individuals (Wroblewski et al. 2010; Bauer and Meyer 2011). The colonization of gastric mucosa by *H. pylori* and its interaction with the epithelium produces a strong chemokine response and attracts large amounts of neutrophils and other immune cells to the site (Dunn et al. 1997; White et al. 2015; Gobert and Wilson 2016). This inflammation continues unless the bacteria are eradicated by therapy, which suggests that resolution processes are limited in this infection. It is known that long-term infection with *H. pylori* produces a robust Th1 immune response and that can control the infection to a certain degree (White et al. 2015). The presence of the pro-resolution factors, such as Treg cells and IL-10, was demonstrated, but this was not sufficient for total clearance and resolution, although these factors are able to reduce the immunopathologies associated with *H. pylori* (Kao et al. 2010; Cook et al. 2014; Hussain et al. 2016). The reduced immune-pathology mediated by IL-10 could be attributed to its pro-resolution effort, but lack of complete *H. pylori* clearance prevents recovery to homeostasis. In addition, human *H. pylori* infection results in a mixed M1/M2 macrophage response, whereas in the mouse infection model a clear M1 polarization is

observed (Quiding-Järbrink et al. 2010; Gobert et al. 2014; Beceiro et al. 2017). This partial pro-resolution in humans may be due to mixed immune effector cell populations, which hinder bacterial clearance and resolution of the infection.

There are a limited number of studies available that describe resolution of *H. pylori* infection, which are either based on observations with vaccinated mice or describe eradication with antibiotic therapy. One early murine study showed that prophylactic immunization reduced colonization levels with several logs 2 weeks after dosage, and after 52 weeks their gastric tissue resembled that of uninfected mice (Garhart et al. 2002). Unimmunized infected mice also reduced colonization levels eventually, but these animals developed gastritis later on. This shows that prophylactic immunization against *H. pylori* doesn't prevent infection but helps to reduce colonization levels and at longer time, either clear the infection or at least resolve the inflammation; however, this effect cannot be excluded to mice specific (Garhart et al. 2002).

Involvement of IL-10 has been studied by infecting *il10*$^{-/-}$ deficient mice. The immune-regulatory role of IL-10 was found to be important in the control of immune responses against *H. pylori*. The IL-10 deficiency enhanced the immune responses and inflammation with a highly significant reduction in bacterial colonization during the early phase, while it reduced gastritis and colonization levels at prolonged infection (Matsumoto et al. 2005). These observations suggest that a reduction in IL-10 producing Treg cells might increase the chance of bacterial eradication and assist resolution in *H. pylori* infection (Matsumoto et al. 2005). It was also shown that infecting *irf1* deficient mice did not result in gastritis or atrophy, despite colonization with very high numbers of bacteria. These mice also failed to produce Th1 and Th2 responses, which correlates with the reduced immunopathology (Sommer et al. 2001). The above data indicate multiple levels of involvement in *H. pylori* induced gastric inflammation and associated pathologies. If we were able to modulate both colonization and inflammation by targeting crucial checkpoints of *H. pylori* infection, it might be possible to clear the infection without the need of antimicrobial drugs.

13 Conclusions

H. pylori currently colonizes about 50% of the world population, although the proportion of infected individuals is decreasing over time. The bacteria are responsible for a significant global health burden, including peptic ulceration and gastric malignancies. More than 30 years of research on the bacteria-host cell interactions taking place during infection have provided amazing insights into the biology of *H. pylori*, with considerable progress being made in the past few years. In this chapter, we have reviewed the interactions of an array of bacterial factors with a wide selection of host signaling modules. Upon the initial intimate contact between bacteria and gastric epithelial cells, specific bacterial factors interfere with selected host receptors and other factors to manipulate the downstream cell signaling. We have highlighted our current understanding how bacterial factors such as VacA, GGT, UreB, NapA, Hsp60, LPS, peptidoglycan and ADP-heptose can hijack host cell signaling modules and downstream signal transduction pathways. It can be presumed that there exists very complex crosstalk between bacterial ligands and their corresponding host cell receptors to influence cellular responses, which ensures chronic colonization and gastric disease development. It remains a highly interesting challenge to further unravel the important actions of *H. pylori* virulence factors, such as translocation of ADP-heptose. In addition, single-nucleotide polymorphisms (SNPs) and other genetic host differences have been reported in multiple cell receptors and immune-regulatory factors including in IL-1β, IL-1 receptor, TLRs, interleukins or TNFα, which also control the clinical outcome of infections by *H. pylori* (see also Chapter "The role of host genetic polymorphisms in *Helicobacter pylori* mediated disease outcome" of this book). In addition to their ongoing uncovering, it can be expected that in the near future additional genetic polymorphisms in

H. pylori populations may also be discovered, now that novel high-throughput sequencing methods have become widely available. This will definitely promote the ongoing assessment and treatment schemes for *H. pylori* infection. Therefore, it appears that *H. pylori* interactions with the host immune system will continue to be an attractive and gratifying subject for future researchers.

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