Immune Evasion Strategies and Persistence of *Helicobacter pylori*

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Abstract *Helicobacter pylori* infection is commonly acquired during childhood, can persist lifelong if not treated, and can cause different gastric pathologies, including chronic gastritis, peptic ulcer disease, and eventually gastric cancer. *H. pylori* has developed a number of strategies in order to cope with the hostile conditions found in the human stomach as well as successful mechanisms to evade the strong innate and adaptive immune responses elicited upon infection. Thus, by manipulating innate immune receptors and related signaling pathways, inducing tolerogenic dendritic cells and inhibiting effector T cell responses, *H. pylori* ensures low recognition by the host immune system as well as its persistence in the gastric epithelium. Bacterial virulence factors such as cytotoxin-associated gene A, vacuolating cytotoxin A, or gamma-glutamyltranspeptidase have been extensively studied in the context of bacterial immune escape and persistence. Further, the bacterium possesses other factors that contribute to immune evasion. In this chapter, we discuss in detail the main evasion and persistence strategies evolved by the bacterium as well as the specific bacterial virulence factors involved.

Contents

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1 Introduction

Despite of eliciting a strong immune response, *H. pylori* can persist lifelong in the gastric mucosa of infected individuals. *H. pylori* infection leads to gastric inflammation, which in most of the cases is asymptomatic, but can progress to a more severe pathology and even gastric cancer (Wroblewski et al. 2010). Therefore, *H. pylori* is considered as class I carcinogen by the World Health Organization (IARC 1994). To survive in the hostile conditions found in the stomach and escape the host's immune response, *H. pylori* has developed different sophisticated strategies, which depend on the presence of certain bacterial virulence factors. Thus, to establish colonization, urease activity as well as flagella are important, while once established in its niche, other virulence factors encoded by the cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), or gamma-glutamyltranspeptidase (GGT) become decisive to shape the host's immune response in order to favor bacterial persistence. These virulence factors can directly alter cellular processes and signaling cascades of host cells or induce changes in the cellular milieu, which also culminate in altered immune cell responses.

Different cell types are involved in the complex immune response toward *H. pylori*, which the bacterium is able to manipulate in order to escape and persist. Thus, epithelial cells and innate immune cells, which constitute the first line of defense against *H. pylori*, are profoundly altered by the bacterium, leading to an ineffective clearance of the pathogen. These changes are decisive to shape the subsequent adaptive immune response, mainly mediated by effector T cells, whose function is also compromised, and contribute to bacterial persistence.

In this chapter, we discuss in detail different strategies employed by *H. pylori* to escape from the host's immune response and persist in the stomach, paying special attention to the bacterial virulence factors involved.

2 Innate Immune Evasion Strategies of *H. pylori*

In order to ensure its persistence, *H. pylori* manipulates the host's innate immune response (Fig. 1), which is mainly driven by gastric epithelial cells as well as gastric-resident innate immune cells or innate immune cells recruited upon infection, including dendritic cells (DCs), monocytes/macrophages, and neutrophils.

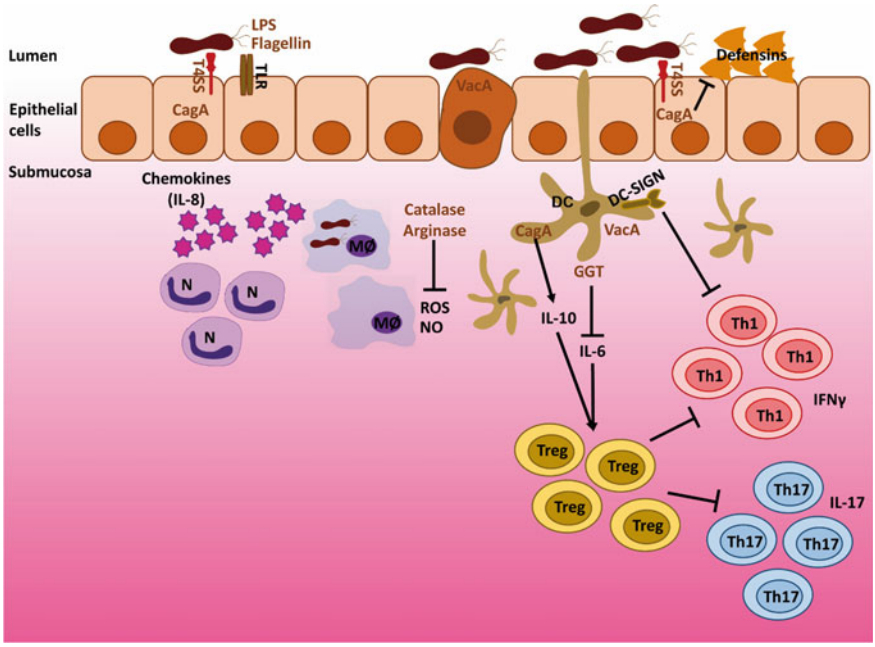

Fig. 1 *H. pylori* evades and manipulates host innate immune responses. Upon *H. pylori* infection, secretion of different chemokines by epithelial cells induces the recruitment of several types of immune cells to the gastric mucosa. However, the bacterium has developed a number of strategies to escape recognition and to dampen immune responses. *H. pylori* LPS and flagellin are weakly recognized by TLRs due to structural modifications, while the secretion of antimicrobial peptides such as defensins by epithelial cells is actively impaired by the virulence factor CagA. The type IV secretion system is important to inhibit phagocytic uptake of the bacterium, which can resist intracellular killing after enfulfement. Thus, the expression of bacterial enzymes as catalase or arginase contributes to the inhibition of phagocytic killing by producing reactive oxygen species (ROS) and nitric oxide (NO). Activation of DC-SIGN signaling in dendritic cells suppresses pro-inflammatory responses, while, by altering the maturation and function of DCs through virulence factors such as CagA, GGT, or VacA, regulatory T cell responses are favored. Abbreviations: N, neutrophil; Mϕ, macrophage; DC, dendritic cell

2.1 *Evasion and Manipulation of Pattern Recognition Receptors*

Epithelial cells and innate immune cells recognize pathogen-associated molecular patterns (PAMPs) by different immune receptors or pattern recognition receptors (PRRs) expressed at specific subcellular compartments. Upon their activation, PRRs induce diverse downstream signaling pathways important for the clearance of pathogens. *H. pylori* has developed a number of strategies to avoid detection by Toll-like receptors (TLRs) and to manipulate and suppress TLR—as well as C-type

lectin receptor (CLR)-mediated signaling. By this, clearance of the bacterium is impaired and persistent colonization of the human stomach is consolidated.

2.1.1 Evasion of TLR Recognition and Manipulation of TLR-Mediated Signaling

TLRs on gastric epithelial cells and immune cells recognize diverse *H. pylori* PAMPs such as lipopolysaccharide (LPS) (TLR2 and TLR4), flagellin (TLR5), and bacterial nucleic acids (TLR8 and TLR9). However, by modulating the expression and structure of surface molecules such as LPS or flagellin, *H. pylori* successfully avoids or dampens recognition by these TLRs (Pachathundikandi et al. 2011, 2015).

LPS is a glycolipid commonly found on the outer membrane of Gram-negative bacteria and composes of three well-defined units: (1) a hydrophobic moiety, lipid A, responsible for the toxic effects; (2) a core oligosaccharide, which contributes to the outer membrane integrity together with lipid A; and (3) the O-antigen, which is a polymer composed of repeating oligosaccharides connected to the core and in direct contact with the extracellular milieu (Whitfield and Trent 2014). By structural modifications in the lipid A and expression and variation of Lewis (Le) antigens terminally exposed on the O-antigen, *H. pylori* has achieved reduced detection by the immune system. *H. pylori* lipid A shows lower biological activity than lipid A of other Gram-negative bacteria (Muotiala et al. 1992; Moran and Aspinall 1998). Uncommon phosphorylation and acylation of lipid A are not only responsible for the reduced endotoxicity of *H. pylori* LPS (Ljungh et al. 1996), but also for its reduced immunogenicity (Moran 2001). Exchange of the 1-phosphate group for a phosphorylethanolamine and removal of the 4′-phosphate group in the *H. pylori* lipid A backbone increase resistance to antimicrobial peptides and reduce recognition by TLRs (Tran et al. 2006; Cullen et al. 2011). These modifications are extremely important for the successful colonization of the gastric mucosa, since *H. pylori* mutants deficient for the phosphatases involved in dephosphorylation of lipid A are not able to colonize mice (Cullen et al. 2011).

The O-antigen of *H. pylori* LPS also contributes to the evasion of bacteria recognition by innate immune cells. The common backbone of this polysaccharide is modified by fucosyltransferases that generate structures mimicking human Lewis antigens and related blood group antigens (Rubin and Trent 2013). Thus, the O-antigen can evade TLR detection since it is not recognized as a foreign molecule but rather as a "self"-antigen. *H. pylori* can adapt to the host by phase variation of its LPS, in which Le antigens of the bacterium evolve to resemble the gastric Le phenotype of the host (Appelmelk and Vandenbroucke-Grauls 2001). Due to the diversity of Lewis antigens expressed in *H. pylori* LPS, the exact role of these molecules in infection and disease is not completely clear. Le^x and Le^y antigens are expressed in almost 80–90% of *H. pylori* strains (Moran 2008). They have been related to bacterial adhesion (Fowler et al. 2006; Heneghan et al. 2000), and more importantly, they contribute to bacterial molecular mimicry to evade immune

responses (Appelmelk and Vandenbroucke-Grauls 2001). It is still under discussion which TLR(s) is/are involved in the recognition of *H. pylori* LPS, since some studies suggest TLR4 as the main sensor (Ishihara et al. 2004; Kawahara et al. 2005), while others support TLR2 as its receptor (Yokota et al. 2007; Smith et al. 2011). Nevertheless, it is clear that the reduced ability of *H. pylori* LPS to bind and activate its receptors contributes to bacterial escape from innate immune responses.

H. pylori flagellin, which is an important bacterial factor for motility and colonization, eludes recognition by TLR5, avoiding pro-inflammatory transcription factor and nuclear factor-kappa B (NF-κB) activation (Pachathundikandi et al. 2016). This is due to a modification in residues 89–96 of the N-terminal D1 domain of *H. pylori* flagellin, important for TLR5 recognition (Andersen-Nissen et al. 2005). In addition, FlaA, the primary flagellar structural component, is not released and is much less pro-inflammatory compared to other bacterial flagellins (Gewirtz et al. 2004).

Apart of having developed mechanisms to escape immune recognition, *H. pylori* has also acquired strategies to actively manipulate TLR-mediated signaling in order to dampen pro-inflammatory responses. For example, activation of TLR2 signaling induces the expression of many anti-inflammatory cytokines via myeloid differentiation primary response gene 88 (MyD88), particularly IL-10 (Rad et al. 2009). In line, infection of *Tlr2*$^{-/-}$ mice indicated an important role for TLR2 signaling in *H. pylori*-induced immune tolerance. *Tlr2*$^{-/-}$ mice were colonized at lower levels than wild-type animals and showed more severe inflammation of the gastric mucosa, reflected by higher levels of interferon (IFN)-γ and lower expression of forkhead box P3 (Foxp3), a transcription factor marking regulatory T cells, IL-10, and IL-17 (Sun et al. 2013).

Another mechanism by which *H. pylori* suppresses gastric inflammation is the activation of TLR9, which was identified as the main intracellular TLR signaling pathway recognizing *H. pylori* DNA in DCs (Rad et al. 2009). Activation of TLR9 signaling is suggested to have anti-inflammatory effects at early phases of *H. pylori* infection. Thus, although *Tlr9*$^{-/-}$ mice showed similar bacterial colonization, they presented a more severe gastric inflammation, which was characterized by increased neutrophil infiltration and upregulation of the expression of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interferon-γ (IFN-γ) (Otani et al. 2012).

2.1.2 Inhibition of CLR-Mediated Signaling

H. pylori can modulate immune responses by interacting with another type of PRR, the C-type lectin receptors (CLRs). CLRs expressed on DCs recognize and bind carbohydrates such as mannose, fucose, or glucan, commonly expressed on bacterial surfaces. Of those, Dendritic cell-Specific Intercellular adhesion *molecule-3*-Grabbing Non-integrin (DC-SIGN) can bind *H. pylori* ligands and is involved in the innate immune response to the bacterium. Specifically, *H. pylori* Le^x and Le^y antigens can interact with C-type lectin DC-SIGN on dendritic cells to block the

development of Th1 cells (Bergman et al. 2004). Further studies confirmed *H. pylori* LPS binding to recombinant human DC-SIGN and showed that addition of fucose or incubation with monoclonal antibodies against Le antigens abolished this binding (Miszczyk et al. 2012). Notably, the fucose residues of the DC-SIGN ligands of *H. pylori* interfere with the signaling complex downstream of DC-SIGN, suppressing pro-inflammatory responses, in contrast to other pathogens expressing mannosylated DC-SIGN that activate pro-inflammatory signaling pathways (Gringhuis et al. 2009). More recently, the expression of macrophage inducible C-type lectin (Mincle) was found to be upregulated in *H. pylori*-infected macrophages. This CLR interacts with Lewis antigens of *H. pylori* and induces an anti-inflammatory immune response, which would also contribute to bacterial immune escape and persistence in the host (Devi et al. 2015).

2.2 *Inhibition of Phagocytosis and Killing by Reactive Oxygen Species and Nitric Oxide*

Bacterial phagocytosis is a central host defense mechanism to eliminate invading bacteria. Upon *H. pylori* infection, different phagocytes such as neutrophils, polymorphonuclear (PMN) lymphocytes, and monocytes are recruited to the gastric mucosa. However, the bacterium successfully inhibits its own uptake by PMNs and monocytes. This antiphagocytic activity depends on different virulence genes, such as *virB7* and *virB11*, and core components of the type IV secretion system (T4SS) (Ramarao et al. 2000a; Ramarao and Meyer 2001) and contributes to bacterial immune escape. Another strategy used by the bacterium to escape phagocytosis involves intrinsic α-glycosylation of cholesterol. Thus, bacteria lacking cholesterol-α-glycosyltransferase HP0421 (Lebrun et al. 2006) were more susceptible to phagocytosis by macrophages and induced a more potent MHC-restricted T cell activation. In addition, HP0421-deficient bacteria were efficiently cleared from the gastric tissue of infected mice (Wunder et al. 2006), indicating that α-glycosylation of cholesterol by *H. pylori* represents a prerequisite for the bacterium to escape phagocytosis, T cell activation, as well as bacterial clearance in vivo.

On the other hand, once engulfed by macrophages, different strategies allow *H. pylori* to survive phagocytosis. *Cag* pathogenicity island (*cag*PAI)-positive and VacA-positive *H. pylori* strains were found to delay actin polymerization and phagosome formation in human and murine macrophages. Once formed, phagosomes underwent clustering and fusion-forming megasomes, wherein *H. pylori* resisted intracellular killing. (Allen et al. 2000). Other strategies to avoid macrophage-mediated bacterial killing include VacA-related arrest of phagosome maturation in association with the retention of tryptophan aspartate-containing protein (Zheng and Jones 2003) and delayed entry and arrest of phosphatidylinositide 3-kinase (PI3 K)-dependent phagosome maturation via synthesis of cholesteryl glucosides (Du et al. 2014).

Survival of *H. pylori* within polymorphonuclear cells (PMNs) is also achieved by disrupting the NADPH oxidase system, which produces reactive oxygen species (ROS) in response to the bacterium. NADPH oxidase assembly is inefficient in the phagosome, and although PMNs containing *H. pylori* produce ROS, they do not accumulate inside the phagosomes but are released to the extracellular space (Allen et al. 2005). *H. pylori* produces catalase and superoxide dismutase, which detoxify ROS and protect the bacterium from the toxic effects of ROS in vitro and in vivo (Spiegelhalder et al. 1993; Odenbreit et al. 1996; Ramarao et al. 2000b; Seyler et al. 2001; Harris et al. 2002, 2003).

Production of nitric oxide by macrophages represents another mechanism to kill bacteria. *H. pylori* urease induces the activation of inducible nitric oxide synthase (iNOS) (Gobert et al. 2002b); however, at the same time, *H. pylori* arginase protects the bacterium against NO-mediated killing by competing with host cells iNOS for the common substrate L-arginine. Thus, macrophages infected with *H. pylori* deficient for arginase efficiently killed the bacterium, whereas *H. pylori* wild type did not show loss of survival under the same experimental conditions (Gobert et al. 2001). The induction of arginase II (Arg2) represents a further mechanism by which *H. pylori* escapes the host innate immune response. Upregulation of Arg2 was detected in *H. pylori*-induced gastritis in humans and mice (Gobert et al. 2002a), and Arg2 activity attenuated *H. pylori*-stimulated NO production by limiting iNOS protein expression in vitro and in vivo (Lewis et al. 2010). Notably, treatment with an arginase inhibitor led to increased iNOS protein expression and NO production in gastric macrophages of *H. pylori*-infected mice (Lewis et al. 2010), confirming that restriction of NO production contributes to *H. pylori* escape from macrophage-mediated killing.

2.3 Inhibition of Antimicrobial Peptides

As part of the innate immune response, epithelial cells generate a number of antimicrobial peptides to protect the gastrointestinal epithelium from invading bacteria. *H. pylori* was reported to induce the expression of human beta-defensin 2 (hBD2) (Hamanaka et al. 2001; Bajaj-Elliott et al. 2002; Wehkamp et al. 2003), hBD3 (Kawauchi et al. 2006) and the amphipathic α-helical cathelicidin LL37 (Hase et al. 2003), which also exhibits a wide spectrum of antimicrobial activity. However, in more recent research, different studies have demonstrated that *H. pylori* is able to manipulate the expression of these antimicrobial substances in order to escape and persist in the gastric mucosa. Thus, the expression of hBD3 is rapidly induced via epidermal growth factor receptor (EGFR)-dependent activation of mitogen-activated protein (MAP) kinase and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling at early stages of *H. pylori* infection. However, during chronic infection, hBD3 is downregulated by *H. pylori* CagA-mediated activation of tyrosine phosphatase Src homology 2 domain-containing phosphatase 2 (SHP-2), which terminates EGFR activation and

downstream signaling, supporting bacterial viability (Bauer et al. 2012). In addition, the *H. pylori* T4SS downregulates the expression of hBD1 through NF-κB signaling (Patel et al. 2013). Interestingly, when analyzing biopsies from infected patients, the same authors observed that patients showing higher bacterial colonization and inflammation showed lower hBD1 expression, whereas they did not detect differences in hBD2 (Patel et al. 2013). Notably, *H. pylori* showed resistance to hDB1, while minimal susceptibility to hBD2 was observed for some strains. On the other hand, hBD3 and LL37 efficiently killed *H. pylori*; however, these two antimicrobial peptides were only marginally detected in the human stomach (Nuding et al. 2013). Therefore, by manipulating defensin expression and developing resistance against these antimicrobial peptides, *H. pylori* can more efficiently colonize the gastric mucosa.

3 Immune Tolerance Driven by *H. pylori* Interaction with Dendritic Cells

DCs are highly specialized antigen-presenting cells, key mediators of the innate and adaptive immune response due to their ability to capture and transfer antigens and regulate T cell responses. DCs can enter the gastric epithelium, where they take up *H. pylori* and its virulence products (Necchi et al. 2009) and as thus shape adaptive immune responses. *H. pylori* has evolved a number of strategies to manipulate DC maturation and cytokine production, skewing them toward a tolerogenic phenotype and therefore instructing a regulatory T cell response that contributes to bacterial persistence (Fig. 2).

In first reports, *H. pylori* undefined secreted factors were reported to inhibit the secretion of IL-12 (Kao et al. 2006), while chronic exposure to the bacterium resulted in increased expression of PD-L1, a member of the B7 family implicated in the inhibition of T cell function, and impaired DC function inhibiting Th1 responses (Mitchell et al. 2007).

Further investigations have implicated the virulence factors CagA, VacA, and GGT in *H. pylori*-induced tolerogenic effects on DCs. In bone marrow-derived dendritic cells (BMDCs), CagA plays an important role in regulating DCs to inhibit $CD4^+$ T cell differentiation toward a Th1 phenotype. Hence, phosphorylation of CagA inside the cells led to the activation of SHP-2, suppressing the activation of serine/threonine protein kinase-1 (TBK-1), the phosphorylation, and nuclear translocation of interferon regulatory factor 3 (IRF-3) and inducing a reduced interferons production by the DCs (Tanaka et al. 2010). In human DCs, once translocated into the cell, CagA induces a DC semi-mature phenotype characterized by low expression of the costimulatory molecule cluster of differentiation (CD86) and the maturation marker CD83 as well as by low expression of the pro-inflammatory cytokine IL-12p70 and increased expression of IL-10, which favors a regulatory T cell response. These changes are orchestrated by the

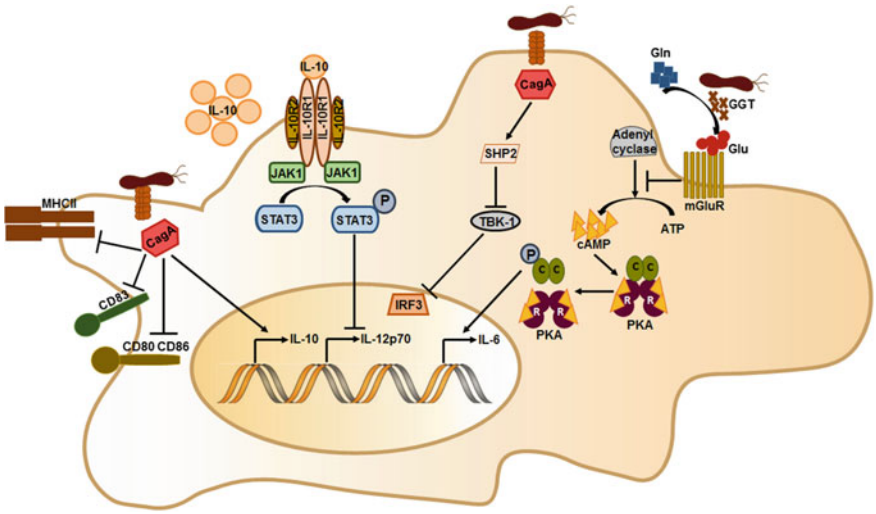

Fig. 2 *H. pylori* induces tolerogenic dendritic cells (DCs). *H. pylori* influences DC maturation and function leading to a tolerogenic phenotype. Translocation of *H. pylori* CagA into DCs dampens the maturation of DCs, which express low levels of MHCII, the maturation marker CD83, as well as costimulatory molecules CD80 and CD86. In addition, it induces high expression levels of the anti-inflammatory cytokine IL-10, which activates STAT3 impairing the expression of pro-inflammatory cytokines as IL-12p70. Moreover, CagA activates SHP-2 leading to the inhibition of IRF3 translocation and IRF-3 interferon-mediated expression. *H. pylori* GGT activity has also a major impact on DC function. Due to *H. pylori* GGT enzymatic activity, glutamine is converted into glutamate that can activate glutamate receptors expressed on the DCs. This leads to the inhibition of cAMP signaling and mediated expression of IL-6. Abbreviations: C, catalytic; Gln, glutamine; Glu, glutamate; JAK, Janus kinase; mGluR, metabotropic glutamate receptor; PKA, protein kinase A; R, regulatory; SHP, src homology phosphatase; STAT, signal transducer and activator of transcription; TBK, TANK-binding kinase

IL-10-dependent activation of signal transducer and activator of transcription factor 3 (STAT3) (Kaebisch et al. 2014).

The *H. pylori* virulence factors VacA and GGT are also important to skew the DC-mediated T cell response toward the bacterium, favoring the development of a predominant regulatory phenotype. In mice, VacA and GGT are required for gastric colonization and DC tolerization in vivo and contribute to neonatally acquired immune tolerance (Oertli et al. 2013). VacA was shown to inhibit DC maturation via restoration of the critical suppressor of DC maturation E2 promoter-binding factor-1 (E2F1) expression (Kim et al. 2011). In human DCs, *H. pylori* GGT also induces a tolerogenic phenotype mainly by repressing the expression of IL-6. This effect is due to the enzymatic activity of GGT, which converts glutamine into glutamate. Glutamate activates metabotropic glutamate receptors expressed on DCs, inhibiting cAMP-mediated regulation of IL-6 expression and thus favoring the expansion of regulatory T cells (Kabisch et al. 2016).

Together, DC tolerization induced by different *H. pylori* virulence determinants has a major impact on the subsequent adaptive immune response to the bacterium, favoring the expansion of regulatory T cells. Indeed, regulatory T cells are massively recruited to the gastric mucosa of *H. pylori*-infected subjects (Lundgren et al. 2003; Cheng et al. 2012) and contribute to bacterial persistence and chronic infection by suppressing effector immune responses (Lundgren et al. 2003; Arnold et al. 2011).

4 Manipulation and Inhibition of Effector T Cell Responses

The adaptive immune response toward *H. pylori* is characterized by the recruitment of $CD4^+$ effector T cells, particularly Th1 and Th17 subsets, which are crucial for the control of the infection and at the same time are implicated in the immunopathological changes resulting from the chronic infection (D'Elios et al. 1997; Bamford et al. 1998; Sayi et al. 2009; Shi et al. 2010; Kabir 2011; Serelli-Lee et al. 2012). As mentioned before, DCs are important regulators and play a key role in defining T cell responses to *H. pylori*. However, in order to persist in the stomach, the bacterium has also developed mechanisms to directly manipulate and inhibit T cells, rendering them hyporesponsive (Fan et al. 1994). In this context, VacA and GGT are the main bacterial virulence determinants impairing effective T cell responses upon infection, although other bacterial factors such as arginase and the *cag*PAI also contribute to dampen T cell proliferation and function (Fig. 3).

VacA can interact with T cells in the lamina propria and enter activated human T cells by binding to β2 integrin (CD18), which associates with CD11a, forming the heterodimeric transmembrane receptor lymphocyte function-associated antigen 1 (LFA-1) (Sewald et al. 2008). VacA uptake is facilitated as *H. pylori* exploits the recycling of LFA-1, and this effect depends on serine/threonine phosphorylation of the β2 integrin cytoplasmic tail by protein kinase C (PKC) (Sewald et al. 2011). Once in the cytoplasm, VacA impairs T cell activation as well as proliferation by different mechanisms. VacA interferes with T cell receptor–IL-2 signaling pathway at the level of calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase. By this, VacA inhibits nuclear translocation of the transcription factor NFAT and prevents transactivation of NFAT-regulated genes specific for T cell immune responses (Gebert et al. 2003). Importantly, VacA suppresses IL-2-mediated cell cycle progression and T cell proliferation through its N-terminal hydrophobic region without affecting IL-2-dependent survival. The N-terminal region of VacA is necessary for the formation of anion-selective membrane channels inhibiting clonal expansion of activated T lymphocytes (Sundrud et al. 2004). Proliferation of T cells is also impaired by the reduction of the mitochondrial membrane potential through *H. pylori* VacA (Boncristiano et al. 2003), while actin rearrangements, as a result of channel-independent activation of intracellular signaling via the MAP kinases

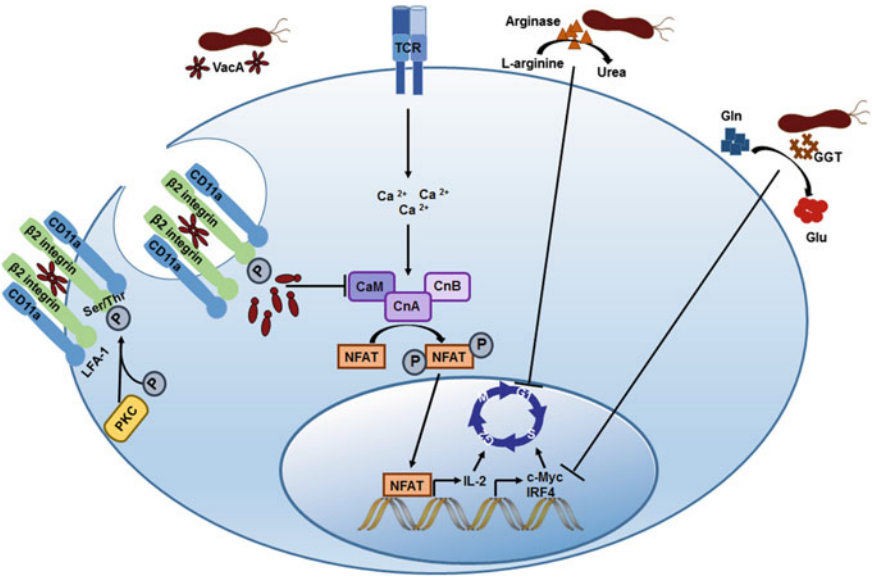

Fig. 3 *H. pylori* inhibits effective effector T cell responses. Effector T cell functionality is altered by *H. pylori* virulence factors. Hexameric VacA binds to the β2 integrin subunit of the LFA-1 receptor, which is internalized after serine/threonine phosphorylation of the β2 integrin cytoplasmic tail by PKC facilitating the uptake of bound VacA. Once in the cytoplasm, VacA prevents nuclear translocation of NFAT by interfering with calcineurin. This leads to impaired IL-2 production and subsequent T cell activation. *H. pylori* GGT also blocks T cell proliferation by depriving cells of glutamine and thereby blocking the expression of transcription factors important for metabolic reprogramming of T cells such as c-Myc and IRF4. Finally, *H. pylori* arginase also contributes to T cell proliferation arrest by depleting L-arginine, which is required for T cell activation and function. Abbreviations: c-Myc, avian myelocytomatosis virus oncogene cellular homolog; CaM, calmodulin; Cn, calcineurin; Gln, glutamine; Glu, glutamate; IRF4, interferon regulatory factor 4; LFA-1, lymphocyte function-associated antigen 1; NFAT, nuclear factor of activated T cell; PKC, protein kinase C; Ser; serine; Thr, threonine

MKK3/6 and p38 as well as the Ras-related C3 botulinum toxin substrate *Rac-specific* nucleotide exchange factor Vav, dampen T cell activation (Ganten et al. 2007).

Another important virulence factor interfering with T cell proliferation and function is GGT. This secreted low molecular weight protein can directly block T cell proliferation by inducing a G1 cell cycle arrest through disruption of rat sarcoma (Ras) signaling pathway (Gerhard et al. 2005; Schmees et al. 2007). More recently, it was shown that *H. pylori* GGT compromises metabolic reprogramming of T lymphocytes by depriving them from glutamine (Wüstner et al. 2015). The expression of IL-2, CD25, and effector cytokines IFN-γ and IL-17 was reduced in the presence of *H. pylori* GGT. Moreover, the expression of the transcription factors c-Myc and IRF4 and signaling cascades as mechanistic target of rapamycin (mTOR) important for T cell metabolic reprogramming were altered by GGT,

indicating that the enzymatic activity of GGT is sufficient to hinder effector T cell responses toward the bacterium and thus contribute to *H. pylori* immune evasion (Wüstner et al. 2015).

Depletion of L-arginine availability by *H. pylori* arginase is another mechanism affecting T cell proliferation. L-arginine is required for T cell activation and function and is depleted by *H. pylori* arginase to produce urea. This induces decreased proliferation of T cells and reduced expression of the chief signal transduction CD3ζ-chain of the T cell receptor, which is necessary for T cell activation (Zabaleta et al. 2004).

*H. pylori cag*PAI-positive strains were found to induce apoptosis of T cells through the induction of the Fas ligand (FasL), limiting host immunity (Wang et al. 2001).

In summary, the inhibition of effector T cells in combination with the development of a regulatory-biased adaptive immune response represents a major mechanism by which *H. pylori* evades the host immune system and persists even though eliciting strong inflammatory responses.

5 Bacterial Plasticity and Immune Evasion

H. pylori is one of the most variable bacterial genus, exhibiting extensive genetic diversity among different strains (Suerbaum 2000). This high level of diversity not only supports adaptation to its host, but at the same time enables *Helicobacter* to avoid several aspects of the immune response. Adhesion molecules of *H. pylori* are not only found in different allelic forms (Pride et al. 2001; Oleastro et al. 2010; Nell et al. 2014), but can also be regulated through on/off mechanisms and were shown to be turned off and replaced by other adhesions after successful colonization (Solnick et al. 2004). These adhesins are also regulated through recombination between various genomic loci. Through this regulation, *H. pylori* avoids immune recognition of important adhesins.

Further, such genomic variability is also observed within a single host (Kraft et al. 2006) and probably reflects adaptation of *H. pylori* to the individual host, including adaptation to specific immune responses. This is reflected by the high variability observed in immune responses among infected individuals. At the humoral level, hardly any antigen is recognized in all infected subjects. Rather, most antibody responses toward a single antigen are detected in a minority of patients. Few antigens, such as CagA, chaperonin GroEL, or flagellar hook-associated protein FliD, are recognized in over 80% of infected individuals (Cover et al. 1995; Nomura et al. 2002; Khalifeh Gholi et al. 2013; Shiota et al. 2014; Pan et al. 2014; Michel et al. 2014). Although far less data are available regarding antigen-specific cellular responses, the same seems to be true here. This could be interpreted as an early adaptation mechanism of *H. pylori*, where protective immune responses lead to rapid epitope changes or selection of subspecies not exhibiting the specific epitope. Over the time, substrains are selected which are weakly recognized.

6 Concluding Remarks

Despite of eliciting strong immune responses, *H. pylori* has, during coevolution with humans, developed a number of strategies to evade the host immune system and persist in the hostile niche that represents the human stomach. Immune evasion and persistence depend on several bacterial virulence factors, which, on the one hand, induce cellular damage and recruitment of immune cells at the site of infection, but, on the other hand, subvert the host's responses in order to create a tolerogenic environment allowing the bacterium to persist. Understanding how *H. pylori* manipulates host immune responses is of foremost importance in order to develop novel and successful therapeutic interventions for the treatment of human populations at high risk of gastric cancer development associated with the infection. This knowledge is also of special relevance in the context of vaccine development against *H. pylori*, since finding adequate antigens to generate vaccine-mediated protection has been extremely challenging so far. Thus, the identification of conserved bacterial proteins well recognized by the host's immune system and important to establish strong immune responses able to clear the bacterium seems imperative for the development of successful vaccine formulations. On the other side, further knowledge on the intricate relations between host and pathogen will be useful to develop strategies to mimic and induce some of the bacterial beneficial effects found in asymptomatic carriers (e.g., asthma protection) while avoiding the deleterious consequences of the infection.

In summary, *H. pylori* is one of the most successful human pathogens able to manipulate the cellular milieu and subvert immune responses in order to ensure its persistence. Although several of the strategies used by *H. pylori* have been identified in the recent years, there are still many lessons to learn from this exceptional bacterium.

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