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Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*

Nina R. Salama[1], **Mara L. Hartung**[2], and **Anne Müller**[2]

[1]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
[2]Institute of Molecular Cancer Research, University of Zürich, Zürich, Switzerland

Abstract

The bacterial pathogen *Helicobacter pylori* has co-evolved with humans and colonizes roughly one half of the human population, but only causes overt gastric disease in a subset of infected hosts. In this Review, we discuss the pathogenesis of this bacterium and the mechanisms it uses to promote persistent colonization of the gastric mucosa, with a focus on recent insights into the role of the virulence factors VacA, CagA and CagL. We also describe the immunobiology of *H. pylori* infection and highlight how this bacterium manipulates the innate and adaptive immune systems of the host to promote its own persistence.

Helicobacter pylori is a highly successful human pathogen that colonizes roughly one half of the world's population. It is typically transmitted orally within families during early childhood and can persist for decades in its preferred niche, the gastric mucosa, despite triggering vigorous innate and adaptive immune responses. *H. pylori* infection causes chronic gastritis, which is asymptomatic in the majority of carriers but is considered a major risk factor for the development of gastric and duodenal ulcers and the two gastric malignancies, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma[1]. In addition to its tight association with cancer, *H. pylori* stands out among other Gram-negative bacterial pathogens in its ability to persist and establish chronic infection.

Contrary to long-held dogma, the stomach is not a sterile organ and is estimated to support a community composed of up to 200 different species[2]. However, when *H. pylori* is present it tends to be numerically dominant and readily visible in gastric biopsy tissue sections as helical rod-shaped organisms covering the gastric epithelial cells and surrounding mucus. Initial colonization depends on bacterial urease activity and cell-shape modulation to penetrate the gastric mucus. Constitutive DNA and protein repair pathways, combined with bacterial genome diversification and attenuation of host cell chemical radical production are now recogized as essential for persistence of the bacterium in this niche. The two known *H. pylori* toxins, VacA and CagA, have been the focus of much work aimed at understanding *H. pylori* virulence. While work on VacA has been reviewed recently[3], we highlight new insights on functional interactions between VacA and CagA and the modulation of immune responses by VacA and another secreted virulence factor, the γ-glutamyl transpeptidase (GGT).

For correspondence: Nina R. Salama, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N, Mailstop C3-168, Seattle, WA 98109-1024, USA. phone: +1 206-667-1540, fax: 206-667-6524, nsalama@fhcrc.org or Anne Müller, Institute of Molecular Cancer Research, University of Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland, phone: +41 44 635 3474, fax: +41 44 635 3484, mueller@imcr.uzh.ch.

Competing interests
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Besides its aresenal of virulence factors, persistence of *H. pylori* is strongly influenced by the ability of the bacterium to evade, subvert and manipulate the host's immune system. This bacterium can evade detection by several innate immune receptors through target modification and it can also subvert other innate recognition pathways through the suppression of downstream signal transduction, while evasion of adaptive immunity is achieved by the modulation of T-cell effector functions.

In this review we discuss the remarkable ability of *H. pylori* to colonize and persist in the hostile environment of the human stomach, through the interplay of several secreted virulence factors and sophisticated manipulation of innate and adaptive immune responses. We also highlight progress on understanding the consequences of persistence for both the bacterium and the host.

Colonization of the gastric mucosa

Escape from the acidic lumen

The stomach is a particularly challenging niche for bacterial habitation. In the lower bowel, which has a neutral or slightly alkaline pH, bacterial density is highest in the lumen; by contrast, the production of gastric acid in the stomach, which results in a pH of 1-2, severely limits luminal colonization. Indeed, *H. pylori* can only survive for minutes in the stomach lumen and must quickly migrate to the gastric epithelial surface[4]. Similar to the intestine, the mucus layer in the stomach forms a physical barrier to bacterial penetration and likely a scaffold for the binding of antimicrobial compounds of the host[5]. Bacterial urease production is required for acid resistance through the localized production of ammonium ions, and flagellar-based motility allows penetration of the mucus (Figure 1)[6]. Furthermore, urease activity facilitates flagellar motility through the mucus layer by changing the viscoelasticity properties of gastric mucins. At low pH, gastric mucins form a gel that effectively traps the bacteria, but urease-catalyzed production of ammonium ions raises the pH to near neutral and the mucus gel transitions to a viscoelastic solution through which *H. pylori* can swim[7, 8]. Regulators of motility, including chemotaxis[9-12] and cell shape[13-15] have been probed to discover additional colonization factors and to better define *H. pylori*'s optimal niche. Helical cell shape is thought to enhance motility through viscous media by a corkscrew mechanism and cell shape mutants that have lost helical twist and/or curvature show attenuated colonization[13-15]. Mutants in several chemotaxis components show altered localization including lower numbers of bacteria in close association with gastric epithelial cells[16] or deeply penetrating the gastric glands[9]. In addition to promoting clearance, the altered localization of chemotaxis mutants correlates with lower inflammation, impaired recruitment of $CD4^+$ T-cells and the absence of a T-helper 17 response[10, 16]. Thus, the intimate association with the gastric epithelium promotes stable infection while simultaneously provoking more inflammation. Higher inflammation correlates with lower bacterial loads[17], which suggests that *H. pylori* must actively manage its interaction with the host epithelium to avoid clearance and persist at this site (Figure 1).

Persistent colonization of the gastric mucosa

H. pylori uses diverse strategies to promote its survival in the face of robust immune responses. All *H. pylori* strains encode proteins important for detoxifying reactive oxygen species (for example, catalase and superoxide dismutase) and *H. pylori* arginase limits NO production by macrophage-, neutrophil- and epithelial cell-derived nitric oxide synthase[18, 19]. Moreover, multiple DNA repair pathways contribute to efficient colonization[20] even while the surrounding host tissue accumulates DNA lesions[21, 22]. Recent work has shown that *H. pylori* strains constitutively express DNA repair proteins such as RecA and thus lack a classic SOS response to DNA damage[23, 24]. Upon DNA

damage, *H. pylori* instead upregulates natural competence which promotes chronic persistence, likely through enhanced genetic diversification[23, 25].

The *H. pylori* genome contains multiple intragenic and extragenic repeat sequences[26]. CagY, which is expressed on the cell surface and is required for the T4SS-mediated translocation of the effector CagA (see below), can undergo recombination between internal repeat motifs that generally preserve the reading frame[27]. During experimental infection of mice and rhesus macaques, CagY variants accumulate that have gained or lost T4SS activity. These results suggest that CagA translocation and the associated biological responses, including inflammation, may have both beneficial and detrimental effects on bacterial persistence leading to selection for both retention and loss of T4SS activity[28].

Among the 60 predicted outer membrane proteins (OMPs), the HOP family shares highly similar or identical sequences at their amino and carboxy termini and includes sevearl known or predicted *H. pylori* adhesins that promote binding to the gastric epithelium[29]. These shared sequences could promote intra- or intergenomic recombination. Sequencing of *H. pylori* HOP loci from human clinical strain collections has revealed likely gene conversion of the Lewis B binding adhesin gene *babA* with *babB* or *babC*[30-33] and of *sabB* with the sialyl-Lewis binding adhesin *sabA* or *omp27*[34-36]. During experimental rhesus macaque or mouse infection, replacement of *babA* with *babB* results in strains that have lost the ability to bind immobilized Lewis B antigens[32, 37]. Additionally, replacement of *sabA* with *sabB* leads to strains expressing two copies of *sabA,* which results in increased binding to sialyl-Lewis antigens on murine gastric tissues[36]. Some alleles of *babA* and *sabA* can undergo phase variation by slipped strand mispairing at dinucleotide sequences in the coding sequences or homopolymeric tracts in their promoters, again leading to either loss or elevated gene expression[38-40]. The carbohydrate antigens bound by these adhesins can be expressed on the cell surface and/or on secreted glycoproteins such as mucin. Furthermore some of these antigens, such as sialyl-Lewis antigens, are induced during inflammation. Phase variation by gene conversion and slipped strand mispairing allows for development of subpopulations with variable adherence properties that could allow the pathogen to evade immune responses or resist shedding. This ability to generate diverse subpopulations may also impact transmission to new hosts.

Secreted toxins of *H. pylori*

VacA and CagA effectors

H. pylori strains actively manipulate host tissues and promote their own persistence through the activity of a number of secreted toxins, some of which are discussed below. Vacuolating cytotoxin A (VacA), which was recently reviewed[3], is a pore-forming toxin that disrupts cell polarity, promotes apoptosis of epithelial cells and inhibits T-cell proliferation and effector functions[3]. The *vacA* gene is carried by all *H. pylori* strains, and sequence variation in several domains of this protein is linked to varying expression levels and cell type specific toxicity, as well as disease severity[3]. Another important toxin is cytotoxin-associated gene A (CagA). Originally characterized as an immunodominant antigen from patients infected with highly virulent *vacA* alleles[41, 42], CagA is translocated into host cells by the Cag type IV secretion system (T4SS) encoded on the *cag* pathogenicity island (PAI)[1, 43]. Strains expressing CagA are associated with an increased cancer risk and transgenic expression of CagA in mice induces gastric carcinoma and other malignancies, which has lead to its designation as a bacterial oncoprotein[44].

CagA-VacA interactions

To act as an oncoprotein, CagA must persist in cells or act in a hit and run manner. CagA has not been readily detected in gastric cancer tissues[45] and thus was suggested to have an

early causative role in cancer progression. It has now been elegantly shown that translocated CagA is degraded by autophagy during infection by *H. pylori* strains carrying the m1 allele of VacA via the ability of this VacA isoform to bind the cell surface receptor low-density lipoprotein receptor-related protein-1 (LRP1)[46]. VacA binding of LPR1 leads to loss of reduced glutathione (GSH) in the cell and increased production of reactive oxygen species (ROS). This in turn activates Akt kinase-dependent degradation of the tumour suppressor p53 and induction of autophagy that degrades CagA. Interestingly, autophagy is not activated in cells expressing a variant form of the CD44 adhesion molecule[46]. These cells have increased intracellular levels of GSH due to activation of xCT, a glutamate-cysteine transporter[47] and therefore do not induce ROS or autophagy upon VacA binding. CD44 is a cell surface marker associated with epithelial cancer stem cells and CagA could be detected in variant-CD44 expressing cells from gastric cancer patients[46]. Paradoxically, tissue changes associated with *H. pylori*-induced gastric carcinogenesis, including development of intestinal metaplasia, were thought to render the stomach less hospitable for *H. pylori* colonization leading to lower colonization loads. However, *H. pylori* was shown to intimately interact with gastric progenitor cells in a mouse infection model[48]. The ability of *H. pylori* to colonize cells having stem cell-like properties and persistance of CagA protein in these cells due to the activiation of xCT and thus impaired autophagy dependent CagA degradation could provide a mechanism for a sustained role of *H. pylori* colonization and CagA in oncogenesis.

Once translocated into host cells, CagA can be tyrosine phosphorylated on EPIYA motifs[49] by Src- and Abl family kinases. These two types of kinases are activated sequentially and in a tightly regulated manner, with Src kinases mediating the initial, preferential phosphorylation of EPIYA-C (and –D) motifs and Abl kinases phosphorylating any EPIYA motif at later time points post-infection[50]. Phosphorylated CagA interacts with SHP-2 tyrosine phosphatase and Csk kinase while unphosphorylated CagA is known to interact with Crk adaptor, c-Met, Grb2, PAR1/MARK and E-cadherin[43]. Collectively, these interactions lead to altered cell signalling and changes in cell polarity, extrusion, motility, proliferation and pro-inflammatory cytokine secretion[1, 43] As discussed below, many of these phenotypes have now been linked to acquistion of nutrients by the bacterium to promote persistance and/or host pathology.

Under standard conditions, CagA expression is not required for stomach colonization, but promotes inflammation in the Mongolian gerbil model[51]. Cag T4SS activity is often lost during murine infection, which complicates efforts to elucidate the pathophysiological roles of CagA during chronic *H. pylori* infection[28, 52, 53]. However, in a polarized cell culture model, CagA promotes increased basolateral uptake and transcytosis of transferrin while VacA drives mislocalization of the transferrin receptor to sites of bacterial attachment to facilitate iron acquisition by the bacterium[54]. In *cagA* mutants, the formation of microcolonies on the apical surface of the cell requires iron supplementation, whereas this is not a requirement for wild-type bacteria, suggesting that CagA- and VacA-dependent cell polarity perturbations confer a nutritional benefit. Consistent with this hypothesis, CagA is required for efficient colonization of Mongolian gerbils under iron-limiting conditions[54]. Thus CagA and VacA collaborate to promote efficient colonization in the iron-limited environment of the stomach and to moderate pathologic effects of CagA.

Ultrastructural insights into CagA secretion

Given the importance of CagA in persistance and pathology, there has been much interest in the mechanisms governing CagA delivery into host cells. Translocation of CagA from the bacterium to the host cell cytosol is mediated by the Cag T4SS. This is a contact-dependent secretion system that forms a large complex spanning the inner and outer membrane, containing a pilus and several ATPases that promote T4SS assembly, pilus formation and/or

CagA translocation[55]. The *H. pylori cag* PAI encodes homologues or paralogues of the prototypical *Agrobacterium tumefaciens* Vir T4SS[56], including the putative VirB7 (CagT), B9 (CagX) and B10 (CagY) inner and outer membrane spanning channel subunits[57], the major (VirB2, CagC) and minor (VirB5, CagL) pilus subunits and several additional *H. pylori* specific *cag* proteins that are required for CagA translocation (for example, CagH and CagI)[58, 59]. Many Cag T4SS components have distinct domain structures from their Vir counterparts. For example, the VirB10 homologue, CagY, is considerably larger (~220 kDa) and contains additional domains composed of repeat regions[27]. Additionally, the three core cell envelope spanning channel subunit homologues, CagY, CagT and CagX, have been suggested to localize to the pilus surface or to the base of the pilus using transmission electron microscopy (TEM)[60, 61]. A later study localized CagL and CagA to the tip of the pilus[62]. CagL was suggested to act as a tip adhesin which binds to α5β1 integrin (the host cell receptor for CagL) through an RGD motif and neighbouring sequences (see insert Figure 1)[62, 63]. CagL binding and α5β1 integrin signalling were found to be required for both pilus extension and CagA translocation. Soluble RGD peptide could partially rescue the CagA translocation defect of a $cagL^{RGA}$ mutant, but not a *ΔcagL* deletion strain suggesting a two-step model, where surface exposed CagL binds and activates α5β1 integrin, partially activating focal adhesion kinase (Fak) and Src kinase, promoting pilus extension. In a second step, pilus associated CagL further stimulates α5β1 integrin, in addition to stimulating the activities of Fak and Src, thereby inducing CagA translocation and ensuring its rapid tyrosine phosphorylation by Src.

The relationship between pilus formation and CagA secretion was further explored by field emission scanning EM (FESEM), which readily detects Cag T4SS dependent pili[64]. This technique confirmed the requirement of CagL for pilus formation and also revealed a hyperpiliated phenotype for *cagH* mutants which, like *cagL* mutants, fail to translocate CagA[64]. A Δ*cagY* mutant produces pili[28], which is surprising because CagL was found to be unstable in a *ΔcagY* mutant in another study[59]. At present, the mechanism by which CagL (or CagA, T, X and Y) becomes surface exposed or incorporated into pili has not been explored. Collectively these data suggest that pilus formation is not sufficient for CagA translocation, that pilus formation can proceed in the absence of at least one core T4SS component, and that there may be CagL-independent mechanisms of integrin activation, of pilus assembly and CagA translocation in some strains. In fact, CagA, CagI and CagY were shown to bind α5β1 integrin *in vitro* and using yeast two hybrid studies[65]. CagA, in particular, shows much higher integrin binding affinity in vitro than CagL; CagA binding is not inhibited by the *Yersinia entercolitica* RGD-containing invasin whereas CagL binding is inhibited, which would indicate that CagA and CagL use different integrin interaction surfaces. Antibodies that prevent integrin switching between a bent and open configuration block CagA translocation[65] and the α5β1 integrin interaction domain of CagA was shown to inhibit CagA translocation when provided as a soluble peptide[66] indicating a complex series of molecular interactions are required for integrin activation and CagA secretion. Further insights into the precise nature of the interactions between CagA, CagL and host interaction partners are beggining to be revealed by structural and molecular evolution studies (see text box 1). .

CagL effector functions

While the CagA translocation defect of *cagL* mutants suggested that CagL has a structural role as part of the T4SS, a number of studies suggest additional functions[67-70]. Studies with purified recombinant CagL revealed that the protein can induce cell spreading and focal adhesion formation in a similar manner to the host extracellular matrix RGD-containing protein fibronectin[69]. CagL activates epidermal growth factor receptor (EGFR) more efficiently than fibronectin, and this was shown to result from RGD-dependent displacement

of ADAM17 (a disintegrin and metalloprotease) from α5β1 integrin, activating ADAM17 protease activity[68]. ADAM17 cleaves and releases surface bound heparin-binding EGF-like growth factor. The resulting activation of EGFR in gastric epithelial cells represses H,K-ATPase activity (and thus diminshes acid secretion) via a repressive NF-κB binding site in the H,K-ATPase promoter. CagL can also bind αvβ5 integrin independently of its RGD motif, which mediates the induction of gastrin[70]. Gastrin is a potent inducer of acid secretion, thus, simultaneous activation of gastrin and repression of the H,K-ATPase could explain the observed hypergastrinaemia and hypochlorhydria during chronic *H. pylori* infection. Finally, CagL RGD-dependent activation of α5β1 integrin activates the pro-inflammatory cytokine interleukin-8 (IL-8) independently of CagA translocation and Nod1 signalling[67], indicating that CagL induces inflammation. Increased risk cancer and ulcer which is associated with the carriage of the Cag PAI, has largely been attributed to CagA but these studies indicate that CagL may be an equally important effector. Furthermore, studies on the evolution of the Cag PAI suggest that additional Cag proteins may directly interact with host proteins via exposure on the cell surface or as novel effectors (see text box 1).

Evasion of innate immune recognition

In addition to the multiple virulence factors that *H. pylori* uses to manipulate the host and ensure its persistence, the pathogen has evolved elaborate strategies to evade and subvert host immune defences, which are key to the success of this pathogen. The first defence barrier against *H. pylori* is formed by mucus-producing epithelial cells lining the gastric mucosa and by innate immune cells that either reside in the gastric lamina propria under steady state conditions or are recruited there during infection. The detection of conserved pathogen-derived molecular structures (pathogen-associated molecular patterns, PAMPs) by epithelial cells and innate immune cells occurs via four distinct classes of innate immune receptors (so-called pattern recognition receptors, PRRs) that differ in their subcellular localization, coupling to downstream signalling pathways and specificity. *H. pylori* has evolved to avoid detection by several types of PRRs that are crucial for the recognition of other Gram-negative enteropathogens.

Evasion and manipulation of TLR and RLR recognition

The best-defined among the four classes of PRRs are the Toll-like receptors (TLRs). TLRs are either surface-exposed on the plasma membrane or localize to endosomes and bind diverse classes of microbially derived PAMPs. Among these are the ligands for TLR4 (LPS), TLR2 (lipoteichoic acid and lipoproteins), TLR3 (dsRNA and polyinosinic:polycytidylic acid), TLR5 (flagellin) and TLR9 (unmethylated CpG). *H. pylori* largely avoids recognition by TLRs, with evasion of TLR4 detection of LPS being the best understood example. *H. pylori* LPS is predominantly tetra-acylated and is 1000-fold less bioactive than the hexa-acylated LPS of *E. coli* [71]. Furthermore, the relative inactivity of *H. pylori* LPS was recently shown to result from the removal of phosphate groups from the 1'- and 4'- positions of the lipid A backbone, which generates LPS that exhibits less negative charge, resists binding by antimicrobial peptides such as polymyxin B and escapes detection by TLRs[72]. The phosphatases responsible for lipid A modification in *H. pylori* have been identified and the respective gene deletion mutants indeed fail to colonize experimentally infected mice[72]. The TLR(s) involved in the residual detection of *H. pylori* LPS remain a matter of debate; whereas several studies utilizing purified LPS have implicated the classical LPS sensor TLR4[73, 74], others suggest that TLR2 is the main sensor of *H. pylori* LPS[75, 76] (Figure 2). A clear interpretation of the published studies is complicated by the fact that they rely on models in which the respective TLR is ectopically expressed, often in the absence of its co-receptor, and the fact that both TLR4 and TLR2 participate in the detection of other, non-LPS-related PAMPs of *H. pylori*[77, 78], which may contaminate LPS preparations.

Another putative *H. pylori* PAMP, flagellin, escapes recognition by TLR5 due to modifications in the N-terminal TLR5 recognition domain[79] (Figure 2). Mutating residues 89-96 of *Salmonella* flagellin to the corresponding *flaA* sequence of *H. pylori* abolishes its recognition by TLR5[80]. Experiments using dendritic cells (DCs) lacking TLRs 2, 4, 7 and 9, or combinations thereof, revealed that the innate immune system recognizes *H. pylori* nucleic acids[77]. Intracellular delivery of *H. pylori* DNA to DCs by lipofection efficiently activates endosomally localized TLR9[77], however the net effect of this activation is anti- rather than pro-inflammatory[81-83] (Figure 2). TLR9 signalling has anti-inflammatory consequences in the early stages of infection in a mouse model[81] and *H. pylori* DNA can even be used therapeutically to treat experimentally induced inflammatory bowel disease in mice[82, 83]. The bioactivity of *H. pylori* DNA may account for the inverse correlation between *H. pylori* colonization and the risk of developing inflammatory bowel diseases[84], which has been attributed to a specific "immuno-regulatory" sequence (TTTAGGG) that appears to be unique to the *H. pylori* genome[82, 83]. *H. pylori* RNA sensing by DCs has been suggested to be mediated by endosomally localized TLR8[77], as well as a cytoplasmic nucleic acid sensor belonging to the RIG-like helicase receptor family (RLRs), RIG-I. RIG-I appears to be required for the detection of 5' triphosphorylated *H. pylori* RNA and the ensuing IRF3/7-dependent induction of type I interferons (IFNs) by DCs[77] (Figure 2). Whether the activation of RIG-I and the *H. pylori*-induced production of type I IFNs has predominantly pro- or anti-inflammatory effects remains to be determined.

The detection of *H. pylori* non-LPS ligands by TLR2 represents another example of how *H. pylori* exploits the immune system for the induction of anti-inflammatory responses. Activation of TLR2 triggers the MyD88-dependent expression of a number of anti-inflammatory genes, most notably interleukin-10[77] (Figure 2). Furthermore, $TLR2^{-/-}$ mice infected with *Helicobacter felis*, a close relative of *H. pylori*, control experimental infections better than wild type mice and develop stronger T-cell responses and T-cell-driven immunopathology[78]. The effects of TLR2 gene deletion are phenocopied by $MyD88^{-/-}$ mice, indicating that the absence of anti-inflammatory signals induced by *Helicobacter* is phenotypically dominant over the simultaneous lack of MyD88-dependent pro-inflammatory signals induced by other TLRs[78].

Suppression of CLR-mediated signalling

In addition to its TLR and RLR ligands, *H. pylori* also harbors ligands for a third class of PRRs, the C-type lectin receptors (CLRs). The best characterized of these are fucosylated ligands that bind to the CLR family member DC-SIGN[85]. In contrast to pathogens such as *M. tuberculosis* and HIV, which express mannosylated DC-SIGN ligands and activate pro-inflammatory downstream signalling pathways, the fucose residues of *H. pylori*'s DC-SIGN ligands actively dissociate the signalling complex downstream of DC-SIGN (consisting of the scaffold proteins LSP1, KSR1 and CNK and the kinase Raf-1) and suppress pro-inflammatory signalling[85] (Figure 2). The differential biological effects of mannosylated and fucosylated DC-SIGN ligands are in line with the proposed role of this PRR in tailoring and fine-tuning adaptive immunity to specific pathogens via the DC-SIGN/Raf-1-mediated acetylation of TLR-activated nuclear NF-κB[86]. Acetylation of the NF-κB subunit p65both prolongs and increases IL-10 transcription to enhance anti-inflammatory cytokine responses[86].

In summary, most of the available data support the conclusion that *H. pylori* avoids the induction of a strong pro-inflammatory response, as well as subsequent adaptive immunity and clearance, through two main mechanisms: the evasion of innate immune detection by pro-inflammatory TLRs and the preferential activation and manipulation of anti-inflammatory TLRs and CLRs. Together, these strategies promote persistence of the organism.

Activation of NLRs and the inflammasome

The heterogeneous cytoplasmic family of Nod-like receptors (NLRs) comprise the fourth and final family of PRRs. NLRs detect a wide range of PAMPs and are also essential for sensing host-derived, damage-associated molecular patterns (DAMPs) that are released upon perturbations of tissue homeostasis[87]. Broadly speaking, NLRs fall into two categories: Nod1 and Nod2 recognize peptidoglycan metabolites and activate the transcription factor NF-κB to induce innate and adaptive immune response genes[88], while most other NLRs promote the assembly of multiprotein complexes called inflammasomes, which activate the cysteine protease caspase-1[89].

Detection of H. pylori *peptidoglycan by Nod1*

Nod1-mediated detection of *H. pylori* peptidoglycan was one of the first PRR-mediated innate immune pathways found to become activated upon *H. pylori* infection[90]. Although initial work indicated that only T4SS-proficient *H. pylori* strains (harboring a functional Cag T4SS) could deliver peptidoglycan and its active metabolite (meso-diaminopimelate-containing N-acetylglucosamine-N-acetylmuramic acid) into the cytoplasm of host epithelial cells[90], it is now clear that outer membrane vesicles (OMVs) from Cag PAI-negative strains of *H. pylori* can also target peptidoglycan to Nod1[91] (Figure 3). Intragastric delivery of OMVs in mice induces innate and adaptive immune responses through a NOD1-dependent but TLR-independent mechanism[91]. The delivery of peptidoglycan by both OMVs and the T4SS occurs at cholesterol-rich lipid rafts[91, 92] (Figure 3). In addition to the initially reported Nod1 signalling pathway resulting in NF-κB translocation to the nucleus[90], Nod1 also activates the transcription factor AP-1 via the ERK and p38-dependent pathways[93]. A direct consequence of Nod1 signalling is efficient killing of *H. pylori* by beta-defensin 2, an antimicrobial peptide produced by Nod1-activated gastric epithelial cells[94]. The idea that *H. pylori*-induced activation of NF-κB depends on Nod1 has recently been challenged by a report showing that introduction of an siRNA specific for Nod1 does not alter the nuclear translocation of the NF-κB subunit p65[95]. This study rather provides evidence for an alternative Nod1-dependent signalling pathway, which activates the IRF3/7 transcription factor to induce the production of type I IFNs that are required for *H. pylori*-specific cytokine and chemokine responses and infection control[95] (Figure 3).

Inflammasome activation by H. pylori

H. pylori harbors one or more ligands that trigger activation of the inflammasome and of caspase-1, a cysteine protease that controls the processing and secretion of two cytokine precursors, pro-IL-1β and pro-IL-18[87]. Like other caspases, caspase-1 is synthesized as an inactive precursor, which becomes auto-proteolytically activated only upon inflammasome assembly. Inflammasome assembly in turn is regulated by ligand binding and subsequent hetero-oligomerization of inflammasome sensors in conjunction with an adaptor molecule (apoptosis-associated speck-like protein containing a CARD, ASC) and pro-caspase-1[87, 89]. Whereas the inflammasome ligands and NLR sensors involved in *H. pylori* detection remain obscure, *in vitro* and *in vivo* studies have demonstrated that caspase-1 becomes activated in DCs upon co-culture with *H. pylori* and that IL-18 and IL1-β are processed and released into the infected gastric mucosa[96, 97] (Figure 3).

There is no evidence to suggest that *H. pylori* actively avoids inflammasome or caspase-1 activation. In fact, mice lacking caspase-1 due to targeted deletion of the corresponding gene clear an experimental infection with *Helicobacter felis* or *H. pylori* more efficiently than wild-type animals, and exhibit more pronounced pathogen-specific T-cell responses and T-cell-driven immunopathology[96]. The explanation for this unexpected observation was provided by mouse strains lacking either IL-18 or its receptor, IL-18R: these mice

phenocopy the effects of caspase-1 gene deletion, i.e. they clear the infection better than wild-type mice due to enhanced T-cell responses, and as a consequence, develop more severe immunopathology.[96, 98] Further analysis revealed that IL-18 is crucial for inducing regulatory T-cell (Treg) responses to *H. pylori* (Figure 3), which in turn restrict excessive effector T-cell activation and promote persistence[98]. Interestingly, IL-1β (the other caspase-1 cytokine substrate) apparently opposes IL-18 function. IL-1R$^{-/-}$ animals lacking the receptor for IL-1β fail to launch *H. pylori*-specific Th1 and Th17 responses, and cannot control an experimental infection (even when vaccinated against *H. pylori* before challenge) and, as a consequence, are protected against even the mildest forms of infection-associated immunopathology[96]. These data corroborate an earlier report showing that stomach-specific expression of human IL-1β is sufficient to induce gastric inflammation and gastric cancer in transgenic mice[99], and also explain why promoter polymorphisms associated with increased steady-state levels of IL-1β predispose carriers to a high gastric cancer risk[100]. Furthermore, the effects of IL-1R gene deletion appear to be phenocopied by *H. pylori*-infected mice lacking the inflammasome adaptor ASC[101]. In conclusion, detection of *H. pylori* via NLRs and subsequent activation of the inflammasome and downstream signalling pathways is crucial for efficient infection control (in the case of Nod1 signalling and inflammasome-mediated IL-1β secretion) and at the same time ensures the restriction of excessive T-cell responses and immunopathological tissue damage (via inflammasome-mediated IL-18 secretion).

Modulation of T-effector cell responses

Suppression of Th1- and Th17-mediated immunity

Experimental infection studies have shed light on the elements of the innate and adaptive immune system required for control of *H. pylori* infections, and in particular for the generation of vaccine-induced protective immunity[17, 102-107]. Whereas B-cells and antibodies are dispensible for *H. pylori* control[103, 104, 107] (at least for the sub-optimal, non-sterilizing reduction in colonization by 1-2 orders of magnitude that is considered the gold standard in the *H. pylori* vaccinology field, see text box 2), it is now clear that CD4$^+$ effector T-cells (not to be confused with the CD4$^+$ Tregs mentioned above), and in particular Th1 and Th17-polarized T-effector cell subsets and their signature cytokines, are critical for proper control of this infection[17, 102, 106]. The same T-cell subtypes have been implicated in promoting the immunopathological changes of the chronically infected gastric mucosa that manifest histologically as atrophic gastritis, compensatory epithelial hyperplasia and intestinal metaplasia in experimentally infected animals and symptomatic human carriers[108, 109].

Two virulence factors have been specifically implicated in the manipulation and inhibition of human T-cells (Figure 4). VacA inhibits T-cell proliferation by interfering with the T-cell receptor/interleukin-2 signalling pathway at the level of the Ca^{2+}/calmodulin-dependent phosphatase calcineurin[110, 111]. VacA prevents nuclear translocation of the T-cell transcription factor NF-AT and its subsequent transactivation of T-cell-specific immune response genes[110, 111] (Figure 4). Follow up studies have since identified β2 integrin (CD18) as the receptor for VacA on human T-cells[112]; β2 integrin associates with CD11a on T-cells to form the heterodimeric transmembrane receptor LFA-1 (lymphocyte function-associated antigen-1). *H. pylori* exploits the recycling of LFA-1 to facilitate VacA uptake[112] in a manner that depends on protein kinase C-mediated serine/threonine phosphorylation of the β2 integrin cytoplasmic tail[113]. The other *H. pylori* virulence determinant implicated in T-cell inhibition is γ-glutamyl-transpeptidase GGT[114, 115]. Similar to VacA, GGT is a secreted factor that blocks proliferation of T-cells through a mechanism that involves the inhibition of cyclin-dependent kinase activity in the G1 phase of the cell cycle by the disruption of the Ras signalling pathway[114, 115] (Figure 4).

Skewing of T-cell responses

Both VacA and GGT also affect T-cell activity in an indirect manner, by promoting the preferential differentiation of naive T-cells into Tregs[116]. Treg differentiation in response to *H. pylori* infection requires the direct interaction of naive T-cellswith 'tolerogenic' DCs that have been exposed to *H. pylori*, either in the gastric mucosa or in the stomach-draining (gastric or mesenteric) lymph nodes[98, 117, 118]*H. pylori*-exposed DCs fail to induce effector T-cell responses of the Th1 and Th17 type *in vitro* and *in vivo*; instead, such DCs preferentially induce the expression of the Treg-specific transcription factor FoxP3, the surface marker CD25 and the anti-inflammatory cytokine IL-10 in naive T-cells [98, 107, 117] (Figure 4). Such peripherally induced Tregs affect the control of *H. pylori* profoundly, as shown in chronically infected patients[119-123] and by animal experiments in which Tregs were systemically depleted in infected hosts[52, 107]. Tregs accumulate in the *H. pylori*-infected human gastric mucosa[119, 121], especially in children[123] and in asymptomatic carriers[122], and effectively suppress *H. pylori*-specific memory T-cell responses[120].

Experimental depletion of Tregs facilitates clearance of *H. pylori* in infected animals[52] and enhances vaccine-induced protective immunity in vaccinated mice[107]. The Treg-facilitated persistence of *H. pylori* requires T-cell-specific expression of IL-10; in fact, IL-$10^{-/-}$ mice and a strain lacking IL-10 expression in the $CD4^{+}$ T-cell compartment are capable of spontaneously controlling experimental infections[52, 78, 124]. The efficient control or even clearance of *H. pylori* in animals invariably comes at the price of enhanced gastric immunopathology (gastritis and epithelial changes such as atrophy and intestinal metaplasia). Interestingly, an analogous observation has been reported for human carriers, which either accumulate large numbers of IL-10-producing, *H. pylori*-specific Tregs and are colonized heavily (asymptomatic carriers), or develop gastric ulcers because their Treg response is inadequate[122]. The *H. pylori*-specific 'tolerization' of DCs, which appears to be a prerequisite for the skewing of T-cell responses (at least in experimental models[98, 117]), requires the activity of both VacA and GGT [116] (Figure 4). While the exact mechanism of VacA- and GGT-specific DC tolerization remains to be elucidated, the newly assigned function of both factors in Treg induction and persistence is consistent with previous reports showing that gene deletion mutants lacking VacA or GGT have colonization defects relative to their parental, VacA/GGT-proficient wild-type isolates[125, 126].

Systemic consequences of immunosuppression

The active inhibition and manipulation of adaptive, T-cell-driven immune responses by *H. pylori* has a variety of consequences for the host. The persistence mechanisms of *H. pylori* are dominant enough to override the protective effects conferred by *H. pylori*-specific vaccination; a challenge infection can only be cleared (or at least strongly reduced) by vaccinated mice if Tregs or DCs are depleted[107]. These observations may partly explain the difficulties and obstacles faced in *H. pylori* vaccine development (see text box 2). An interesting side effect of *H. pylori*-specific immunomodulation and manipulation is evident in Western societes from which *H. pylori* is gradually disappearing due to reduced transmission rates, the frequent use of antibiotics in childhood and generally improved sanitation conditions[127]. In these populations, the incidence of allergic asthma, other allergic disease manifestations and chronic inflammatory diseases is steadily increasing and an inverse association with *H. pylori* colonization has been documented for allergic asthma[128-132] and inflammatory bowel diseases[84] (see text box 3). While the exact mechanisms underlying this inverse association remain to be elucidated, the idea that *H. pylori*-induced immune regulation and manipulation are causally linked to protection from such immune disorders is compelling (see text box 3). The fact that Tregs isolated from *H. pylori*-infected mice are sufficient to protect naive recipients against allergen-induced asthma in adoptive transfer models argues in favor of Treg-mediated cross-protection

against allergen-specific immune responses[98, 133]. Further work in this area is urgently needed to shed more light on the intricate interactions of this extraordinarily well-adapted, persistent pathogen with the host adaptive immune system.

Conclusions and future perspectives

The work summarized in this Review outlines how *H. pylori* uses a combination of virulence factors and immune subversion and manipulation mechanisms to colonize and persist in the challenging environment of the gastric mucosa. Experimental work in recent years has elucidated exciting details on the structure and function of the type IV secretion system, the pleiotropic effects of CagA delivery, the CagA-independent effects of the secretion system and the newly discovered function of the extracellular effector CagL. The role of cell shape and chemotaxis in persistent colonization is now well documented with respect to the genes involved. Progress in other areas, in particular *H. pylori*-specific vaccine development, has suffered from setbacks in phase I clinical trials and the lack of continuous industry support. The necessity of overriding the bacteria's persistence strategies has been identified as a major challenge in *H. pylori*-specific vaccine development. More and more interest in the field has shifted to gaining a better understanding of the benefits, suggested from epidemiological studies, that the infection may bestow on the large majority of asymptomatic carriers, and experimental evidence has been forthcoming to support such claims. In particular, it is now becoming increasingly clear that the virulence factors deployed by *H. pylori* and the mechanisms that are exploited to override T-cell-driven immunity and to ensure persistent infection have systemic immunomodulatory effects that likely explain the benefits of the infection to asymptomatic carriers. The molecular mechanisms allowing *H. pylori* to suppress T-cell activation via production of VacA and GGT, and to skew T-cell responses towards regulatory T-cells, are increasingly well understood.

Other aspects of the *Helicobacter*-host interaction have received surprisingly little, if any, attention; these include the specifics of inflammasome activation by *H. pylori* and of innate immune activation by *H. pylori* in general, the molecular basis of its host species specificity, and the relative (or perhaps additive) contributions of its direct and indirect (inflammation-mediated) carcinogenic properties to gastric cancer development. Another important aspect of *H. pylori* biology that has been largely ignored relates to its transmission. While several independent, mostly older, studies indicate that mothers serve as the predominant source of their children's *H. pylori* infection, the transmission route remains unclear. Also, very little is currently known about the vast differences in gastric cancer risk of (often closely related, and physically close) human populations, which is likely to be influenced by human genetic predisposition, population ecology and behaviour. In summary, many of the peculiarities that set *H. pylori* apart from other Gram-negative enteropathogens remain underexplored and deserve further work.

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Biography

Anne Müller

Anne Müller studies the pathogenesis of *Helicobacter*-induced gastric cancer and gastric lymphoma. Additional research interests include *H. pylori* virulence factors, persistence mechanisms and innate immune recognition. She received her Ph.D. in 2000 at the Max Planck Institute of Infection Biology, Berlin, Germany, and then moved to the laboratory of Stanley Falkow at Stanford University, California, to pursue postdoctoral research on *Helicobacter*-host interactions. She has been an independent researcher and Faculty member at the Institute of Molecular Cancer Research, University of Zürich, Switzerland, since 2006.

Mara L. Hartung

Mara L. Hartung studied biology at the University of Tübingen, Germany. She is currently pursuing her doctoral studies at the Institute of Molecular Cancer Research, University of Zürich, Switzerland. Her research focuses on investigating the mechanism of DNA damage inflicted on gastric epithelial cells by *H. pylori* and the implications of this process for gastric carcinogenesis.

Nina Salama

Nina Salama investigates *H. pylori* pathogenesis with a focus on bacterial determinants that promote persistent colonization. Current insterests include generation and consequeces of helical cell morphology, genetic variation during chronic colonization, mechanisms of transmission and *H. pylori* interactions with other bacteria and tissues in the upper gastrointestinal tract. She received her Ph.D. in 1995 at the University of California, Berkely, and then moved to the laboratory of Stanley Falkow at Stanford University, California, to pursue postdoctoral research on *Helicobacter pylori* bacterial genetics. She has been an independent research and Faculty member at the Fred Hutchinson Cancer Research Center and Affiliate Professor of Microbiology at the University of Washington in Seattle Washington, since 2001.

Glossary Terms

mucosa-associated lymphoid tissue (MALT) lymphoma	A cancer originating from marginal zone B cells of mucosa-associated lymphoid tissue. *H. pylori* infection-associated MALT lymphomas can often be treated with *H. pylori* eradication therapy which eliminates the antigenic driver of proliferation.
gastric adenocarcinoma	A malignant epithelial tumor originating from the glandular epithelium of the stomach.
Type IV secretion system (T4SS)	A Gram-negative bacterial contact dependent specialized secretion system evolutionarily related to the *Agrobacterium tumefaciens* transforming plasmid pilus. These multi-subunit molecular machines deliver proteins and/or protein-DNA complexes from the bacterial cytoplasm, across the bacterial cell wall (inner membrane, periplasm, outer membrane), and across a target eukaryotic cell plasma membrane to the target cell cytosol.
SOS response	A coordinated transcriptional response to DNA damage extensively characterized in *E. coli* which is initiated by activation of RecA through binding to damaged DNA, resulting in inhibition of cell cycle progression and increased expression of DNA repair proteins and mutagenic DNA polymerases.

natural competence	Ability to take up extracellular DNA and recombine it into the bacterial chromosome.
gene conversion	An intragenomic recombination event where gene sequences at one position in the genome are replaced with homologous sequence encoded at a different location.
slipped strand mispairing	A mutagenic process that can occur during DNA replication of dinucleotide or homopolymeric repeats due to mispairing of complementary bases.
pathogenicity island	Genomic islands that often encode virulence determinants and are typically acquired by horizontal transfer.
cell polarity	Epithelial cells form connections between cells that restrict free diffusion both within the membrane and between cells resulting in an apical surface oriented towards the lumen and a baso-lateral surface oriented towards the underlying submucosa.
polarized cell culture model	a monolayer of cultured epithlial cells that form tight junctions, usually grown on filters that allow the apical and basolateral compartments to be accessed separately.
transcytosis	Movement of molecules across an epithelial cell, including uptake from the apical compartment space and delivery to the baso-lateral compartment.
transmission electron microscopy	A microscopy technique that transmits a beam of electrons through an ultra thin specimen and is capable of imaging at much higher resolution than light microscopy.
field emission scanning EM	An electron microscopy technique where an image is produced by scanning the sample with a focused beam of electrons using a field emission gun which generates a smaller diameter beam.
evolved VgrG subunits	Type VI secretion system subunits that have both a structural role in formation of the secretion apparatus and an effector function upon delivery into host cells.
fibronectin	an extracellular matrix protein that bind integrins.
sterilizing immunity	Protective immunity that results in complete clearance of the pathogen, i.e. to below the detection limit, as opposed to a mere reduction in colonization levels.
adjuvants	pharmacological or immunological agents that are added to vaccine formulations to improve their immunogenicity.
gastroesophageal reflux disease (GERD)	also called acid reflux disease, refers to mucosal damage caused by stomach acid flowing back from the stomach into the esophagus
Methacholine	muscarinic receptor agonist that is clinically used to diagnose bronchial hyperreactivity, a hallmark of asthma and of chronic obstructive pulmonary disease. The methacholine challenge test involves inhalation of aerosolized methacholine, which leads to bronchoconstriction.

pathogen-associated molecular patterns (PAMPs)	molecules with shared molecular motifs that are conserved among certain classes of microbes and detected by innate immune receptors; examples include LPS, lipoteichoic acid, flagellins, double stranded RNA and hypomethylated CpG dinucleotides.
pattern recognition receptors (PRRs)	surface-localized, endosomal or cytoplasmic receptors expressed by innate immune cells that recognize pathogen-associated or damage-associated molecular patterns; PRRs are classified according to their ligand specificity, function, localization and/or evolutionary relationships.
dendritic cells (DCs)	innate immune cells of characteristic morphology that serve as antigen- presenting cells; immature DCs constantly sample their environment for invading microorganisms, which are phagocytosed and their antigens processed and presented to T-cells upon migration to lymphoid organs.
type I interferons	synonymous with IFN-α and IFN-β; cytokines that are expressed by many leukocytes, with especially large quantities derived from plasmacytoid dendritic cells. They have an important role in anti-viral defenses through their activity on NK cells and macrophages.
MyD88 (Myeloid differentiation primary response gene 88)	cytplasmic adaptor protein that couples ligand-activated TLRs (except TLR3) to downstream signaling pathways ultimately leading to NF-κB activation.
damage-associated molecular patterns (DAMPs)	also referred to as danger-associated molecular patterns, are factors produced under inflammatory conditions of non-infectious origin or by damaged or stressed tissues. Examples include proteins (e.g. heat shock proteins, S100 proteins), other macromolecules (extracellular or cytoplasmic DNA), ATP, adenosine and uric acid crystals.
inflammasomes	large cytoplasmic multiprotein complexes that sense microbial infections or danger molecules and initiate auto-proteolytic cleavage of caspase-1, and the subsequent processing and release of pro-inflammatory cytokines, namely IL-1β and IL-18. Various types of inflammasomes can be distinguished based on the Nod-like receptor involved in their activation.
outer membrane vesicles (OMVs)	also referred to as ‘blebs’, are shed from the outer membrane by Gram-negative bacteria during normal growth, and have been reported to enter and transport virulence factors into host cells. OMVs contain numerous components of the bacterial cell wall, including peptidoglycan.
regulatory T-cell (Treg)	a subpopulation of mostly $CD4^+$ T cells that suppress immune responses by other cells, maintain self-tolerance and immune homeostasis, and prevent auto-immunity and excessive immunopathology. The lineage-defining transcription factor of Tregs is FoxP3.

polarized T-effector cell subsets	upon activation via their T-cell receptor, T cells may differentiate into one of several T-helper subtypes, including Th1, Th2 and Th17 cells, which differ in their lineage-defining transcription factors and cytokine expression profiles. The cytokine cocktail provided by the antigen-presenting cell during T-cell activation determines Th subset polarization/ differentiation.
Ras signalling pathway	Ras is the prototypical member of the Ras superfamily of small GTPases; Ras-regulated signalling pathways control a variety of cellular processes including actin cytoskeletal rearrangements, proliferation, differentiation, cell adhesion, apoptosis, and cell migration. Ras and Ras-related proteins are often deregulated in cancers.
naive T-cells	T-cells that have not yet come in contact with their cognate antigen

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Text box 1: Structural and evolutionary insights on Cag proteins

A combination of NMR, X-ray crystallographic, biochemical and localization studies have revealed that CagA, which can form a dimer, has a novel elongated structure that directly facilitates its interactions with α5β1 integrin, phosphatidyl serine (PS) in the cell membrane and the Par1 kinase, as well as inter- and intramolecular interactions with itself[134] (see insert of Figure 1). The ordered N-terminal 70% of the protein contains three domains. In addition to the integrin interacting sub-domain (D2'), Domain II contains a basic patch that includes a previously defined PS interaction surface[135], which causes external accumulation of PS at sites of bacterial attachment and is necessary for CagA translocation. After translocation, this same patch is required for CagA membrane tethering in polarized cells (as shown by transfection studies) and Par1-mediated disruption of polarity[135]. Domain III can form an intramoecular interaction with the intrinsically disordered C-terminal 30% of the protein that includes the Src Homology 2-binding EPIYA motifs and the Par1 interacting Cag multimerization (CM) motif. Interestingly, this intramolecular interaction appears to restrain this domain in a lariat structure that apparently stabilizes interactions with Par1 through reduced turnover, enhancing CagA-dependent induction of cell motility[134]. Mutational studies are beginning to define the specific molecular interactions predicted from these structural studies.

Additional insights into the precise residues that may be important for interactions between CagA and other potential effectors and their host targets comes from molecular evolution and disease association studies. Evaluation of the functional consequences of transient and fixed mutations indicates continuous selection for maintenance of Cag PAI function in spite of frequent loss or inactivation in many *H. pylori* strain populations, suggesting a mixture of positive and negative selection pressures on this virulence determinant[136]. European origin of strains correlates with increased risk of gastric cancer, higher inflammation and accumulation of host DNA damage found in the mountain region (high risk) compared to coastal region (low risk) in Columbia[137]. One study found an association between two linked nonsynonymous SNPs near the RGD motif of CagL and gastric cancer, and higher α5β1 integrin expression in the corpus region of the stomach in a Taiwanese population[138]. Another study found an association between two synonymous SNPs in the CagE ATPase and gastric cancer in a population from Venezuela and Columbia[139]. Analysis of positive selection, which indicates an accumulation of diversifying mutations, revealed strong signals for the known effector proteins CagA and CagL, the surface exposed protein CagY and an uncharacterized protein CagQ[136]. This elevated rate of diversification could result from immune pressure or interaction with host proteins that are polymorphic in different hosts. Further analyses of these variants in view of the recent stuctural data combined with functional studies and replicate associations in other populations will further expand our understanding of *H. pylori* pathogenesis and strain dependent disease risk.

Text box 2: Progress and obstacles in *H. pylori*-specific vaccine development

Efforts to develop a *H. pylori*-specific vaccine began in the early 1990's with the recognition that infection with this bacterium is the most important cause of peptic ulcer disease and a strong risk factor for gastric cancer. Prophylactic immunization, especially during infancy or early childhood, was projected as recently as 2009 to be cost-effective in the U.S. (with respect to dollars spent per quality-adjusted life years[140]), despite the documented gradual loss of *H. pylori* from Western populations[127]. However, the results of *H. pylori* vaccine development efforts, both preclinical and early clinical, have been disappointing to date. Sterilizing immunity is rarely achieved, even in animal models, and there is no consensus on the delivery route, adjuvants and choice of antigens. The most promising preclinical results have generally been obtained with vaccination strategies aimed at inducing protective T-cell-mediated rather than humoral immunity, with local gastric Th1 and Th17 responses representing reasonably good correlates of, and prerequisites for, protection (reviewed recently in [141]).

H. pylori antigens ectopically expressed in *Salmonella enterica* vaccine strains, whole cell *H. pylori* extracts, and multi-component, parenterally or mucosally delivered recombinant vaccines have all been used successfully in mice (for recent comprehensive review articles see[141, 142]). *H. pylori* antigens with documented immunogenicity in rodents include the urease enzyme, CagA, VacA, catalase, neutrophil-activating protein (NAP) and heat shock proteins; these can be delivered by various mucosal routes such as orogastric, intranasal, sublingual and rectal[141, 142]. Despite the strict limitation of *H. pylori* to its gastric niche, systemic immunization via the intraperitoneal or subcutaneous routes can be as effective as mucosal vaccination[141, 142]. In contrast to most other vaccines, *H. pylori*-specific immunization generates prophylactic as well as therapeutic immunity in rodent models. Persistence mechanisms employed by *H. pylori* to overcome and subvert adaptive immunity have been identified as crucial obstacles precluding sterilizing immunity[107]; vaccination strategies may therefore need to be designed to bypass or override the host immunoregulatory response.

Two recently conducted phase I clinical trials in human volunteers showed antigen-specific humoral and cellular responses[143, 144], but did not confer satisfactory protection against challenge infection[143]. In one trial, intramuscular immunization with three recombinant antigens (CagA, VacA and neutrophil-activating protein- NAP) adjuvanted with alum induced responses to some or all antigens in the majority of volunteers irrespective of the exact doses and immunization schedules; T-cell responses were observed only against CagA and VacA, but were detectable as late as 24 months post primary vaccination and thus indicative of T-cell memory[144]. Oral immunization with live *Salmonella enterica* serovar Typhi Ty21a expressing *H. pylori* urease or HP0231 provided evidence that the clearance or reduction of a challenge infection requires T-cell mediated immunity, yet the study failed to demonstrate improved infection control in the vaccinated group relative to the non-immunized (but challenged) volunteers[143]. In conclusion, whereas rodent models of *H. pylori*-specific vaccination have revealed useful antigens, adjuvants and delivery routes, the ultimate proof of immunogenicity and protective immunity in humans remains elusive. The continued support from private sector initiatives is widely viewed as being essential to promoting *H. pylori* vaccine development also in the future[142].

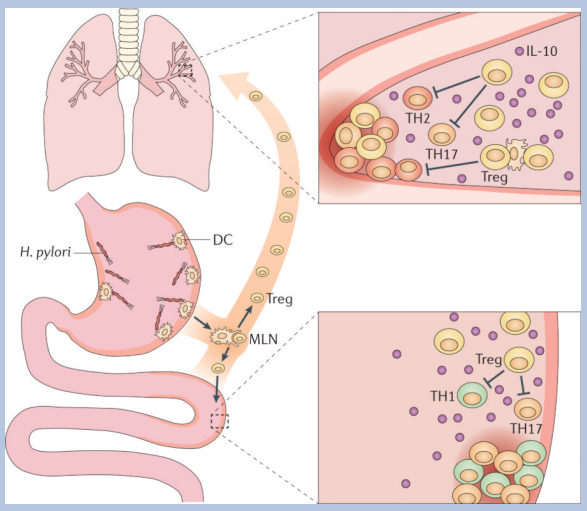
Text box 3 *H. pylori*-mediated protection against allergic and chronic inflammatory disorders

H. pylori is an ancient member of the human indigenous microbiota[145], but began to disappear from individuals in developed countries in the twentieth century[127]. This has led to diminishing rates of peptic ulcer disease and gastric cancer[146]. Coincident with the decline in *H. pylori* colonization rates, especially during the second half of the last century, the incidence of allergic asthma and other allergic disease manifestations has reached epidemic proportions in large parts of the developed world. Cross-sectional studies have documented that the two phenomena are inversely correlated, with *H. pylori* carriers exhibiting a decreased risk of developing childhood- or early-onset allergic asthma, rhinitis and atopic dermatitis than the non-infected population[129, 130, 131, 147]. A large meta-analysis documented a similar inverse relationship between *H. pylori* status and the risk of developing either of two inflammatory bowel diseases, Crohn's disease and ulcerative colitis[84].

A limited amount of experimental evidence from animal studies is now available to support both of these observations (summarized in the figure). In a model of acute *Salmonella typhimurium*-induced intestinal inflammation, experimental co-infection with *H. pylori* suppresses *Salmonella*-specific Th17 responses as well as cecal *Salmonella*-induced inflammation, likely via increased production of IL-10[148]. Similarly, the administration of a single dose of *H. pylori* DNA reduced sodium dextran sulfate (DSS)-induced colitis, in both acute and chronic experimental settings[83]. With respect to asthma, murine infection with *H. pylori* efficiently prevents ovalbumin- or house dust mite-induced allergic airway inflammation[133]. Infected mice are protected against airway hyper-responsiveness (measured after methacholine exposure), as well as tissue inflammation and goblet cell metaplasia, and exhibit reduced pulmonary and bronchoalveolar infiltration of eosinophils, Th2 cells and Th17 cells[133]. Asthma protection could be attributed to *H. pylori*-induced, highly suppressive Tregs, which accumulate in the lungs of infected mice and block allergen-specific effector T-cell responses (see figure). In line with thisobservation, the adoptive transfer of mesenteric lymph node (MLN)-derived $CD25^+$ Tregs from infected donors is sufficient to protect naive recipients against asthma[133]. The induction of *H. pylori*-specific Tregs with suppressive properties in turn involves tolerogenic DCs[98], which presumably encounter *H. pylori* or its tolerizing persistence determinants in the gastric mucosa and then home to the MLNs for T-effector and Treg-cell priming. The suppression of both asthma and colitis is believed to require (DC and/or Treg-derived) IL-10 (see the figure).

Figure for text box 3. *H. pylori* exerts systemic immunomodulatory effects

H. pylori exclusively inhabits the gastric mucosa but it also has systemic immunomodulatory effects that manifest in the airways and lower gastrointestinal tract. Tissue-resident DCs can sample *H. pylori* antigens in the gastric mucosa and subsequently migrate to the stomach-draining and mesenteric lymph nodes (MLNs), where they prime T-cell (in particular Treg) responses. Alternatively, soluble antigens can be transported via the lymph to the MLNs for presentation by resident DC populations. MLN-derived, *H. pylori*-specific Tregs enter the circulation and accumulate not only in the gastric mucosa, but also at other mucosal surfaces of the body, such as those of the airways and lower bowel. According to current models, 'pathogenic' effector T-cell populations (allergen-specific Th17 and Th2 cells and colitogenic Th1 and Th17 cells) are suppressed by *H. pylori*-specific Tregs via soluble mediators (such as IL-10) and contact-dependent mechanisms. Th1/2/17, T-helper cell subsets; Treg, regulatory T-cell.



Summary (online only, 6 bullet points, 1-2 sentences each)

-*Helicobacter pylori* persistently colonizes the gastric mucosa of humans, infecting roughly every other individual worldwide. The prevalence of *H. pylori* is decreasing in most parts of the developed world due to improved sanitation, reduced transmission and more frequent use of antibiotics in childhood.

-As a consequence of the loss of *H. pylori* from Western societies, the incidence of peptic ulcer disease and of gastric cancer has decreased continuously in populations affected by this trend. At the same time, allergies and chronic inflammatory disorders have become more common; epidemiological and experimental evidence suggests an inverse causal association between the loss of *H. pylori* and the rise in these immunological disorders.

-The initial colonization of the hostile environment of the gastric mucosa requires specific adaptations: these include flagella-based motility, production of urease enzyme, chemotaxis and helical cell shape.

-To establish persistent infection, *H. pylori* has evolved to avoid recognition by pattern recognition receptors of the innate immune system, and to preferentially activate receptors coupling to anti-inflammatory signaling pathways. Its pathogen-associated molecular patterns are substantially less bioactive than those of related gram-negative enteropathogens.

-Another *H. pylori* persistence strategy involves the prevention and manipulation of T-cell-mediated adaptive immunity. Specific virulence factors are produced by all strains of *H. pylori* to block T-cell activation, proliferation and effector functions, and to preferentially induce T-regulatory over T-effector responses. Asymptomatic carriers are more likely to preferentially generate Treg responses and to harbor dense *H. pylori* populations than patients with peptic ulcer disease.

-*H. pylori* causes gastric disease due to its production of vacuolating cytotoxin and a pathogenicity island-encoded type IV secretion system; both virulence determinants act in concert to promote the production of pro-inflammatory cytokines, to disrupt cell polarity and to cause tissue damage. The advantage for the bacteria of the production of these virulence determinants remains poorly understood, and may involve improved iron acquisition or enhanced transmission.

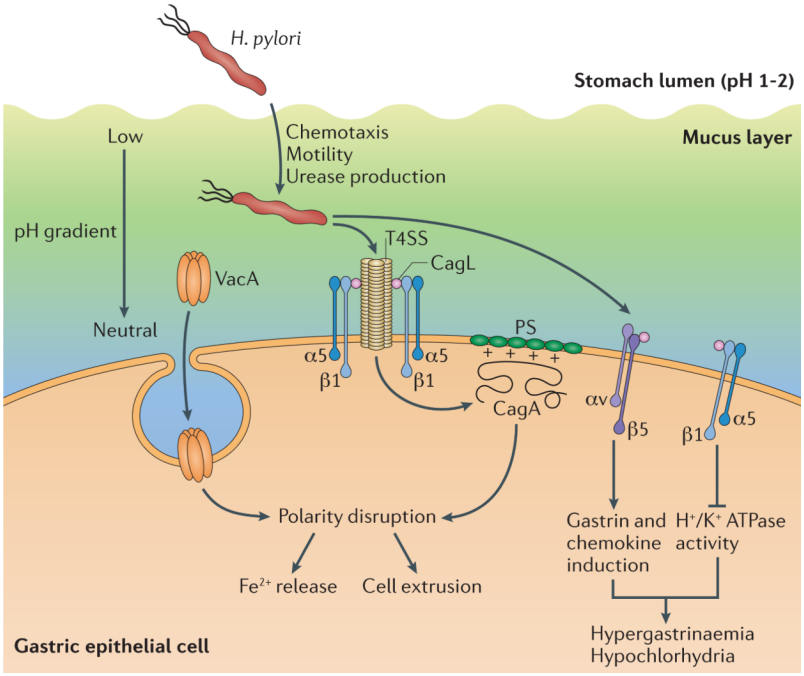


Figure 1. *H. pylori* colonization and persistence factors

During initial infection of the stomach lumen, urease-dependent ammonia production locally raises the pH, which promotes bacterial survival and solubilizes the mucus gel to facilitate bacterial motility. Chemotaxis(driven by pH and possibly other gradients) and helical rod shape promote flagellar-based motility away from the acidic lumen to *H. pylori*'s preferred niche, on and adjacent to gastric epithelial cells. SabA, BabA and other variably expressed adhesins may shift the balance to cell-associated bacteria. Inset: cell-associated bacteria alter gastric epithelial cell behavior through VacA, CagA and CagL which all have multiple cellular targets. This includes CagA and VacA dependent disruption of cell polarity that can promote iron acquisition or cell extrusion, CagA and CagL dependent induction of chemokines and/or the gastric hormone gastrin, CagL dependent inhibition of acid secretion by the H+/K+ ATPase, and affects on proliferation, apoptosis and differentiation mediated by all three effectors. As noted in the main text, in addition to CagL (depicted), CagA and CagY have also been shown to bind α5β1 integrins although the precise interaction surface remains to be determined. fla, flagella; che, chemotaxis; ure, urease; T4SS, Cag Type IV secretion system; PS, phosphatidylserine; α5β1 and αvβ5, indicated integrin subunits.

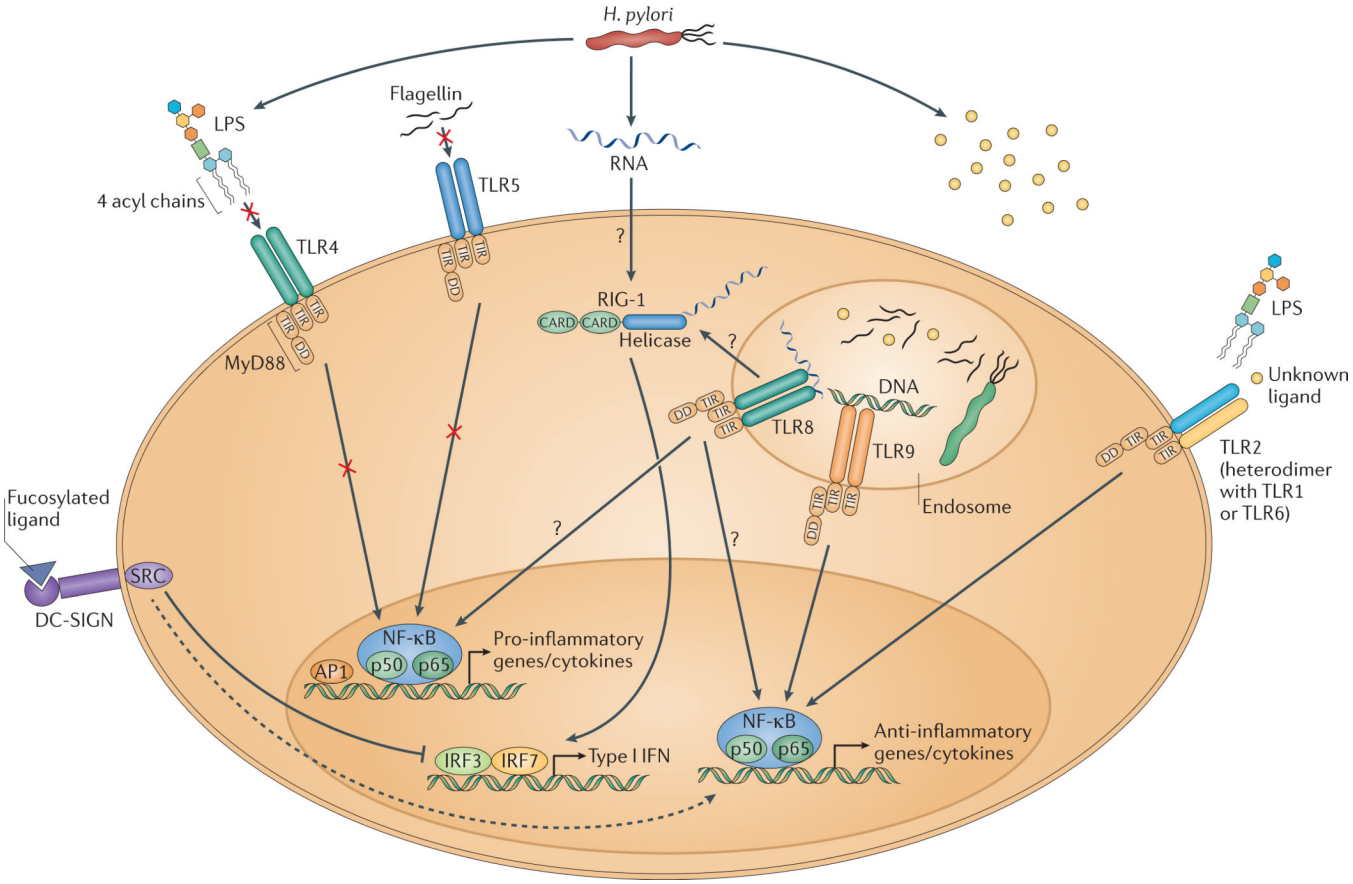


Figure 2. *H. pylori* subversion of innate immune recognition

H. pylori harbors PAMPs that have evolved to evade detection by pro-inflammatory TLRs. *H. pylori* expresses tetra-acylated LPS, which is less bioactive than the hexa-acylated form typical of other Gram-negative pathogens due to specific lipid A modifications that prevent detection by TLR4. *H. pylori* flagella are not detected by TLR5 due to mutations in the TLR5 binding site of flagellin. The bacterium's DNA, as well as an as yet uncharacterized PAMP (and possibly *H. pylori* LPS) are detected by TLRs 9 and 2, respectively; these TLRS predominantly activate anti-inflammatory signalling pathways and anti-inflammatory IL-10 expression. 5‘ triphosphorylated RNA is detected by the RLR RIG-I, which activates the transcription factors IRF3 and IRF7 to induce type I IFN expression, and is potentially detected also by TLR8 in endosomes. *H. pylori*'s fucosylated DC-SIGN ligands suppress activation of the signalling pathways downstream of this CLR and activate anti-inflammatory genes. Please note that not all depicted TLRs, RLRs and CLRs are necessarily expressed by the same cell type; only one generic cell type is shown here for simplicity. DD, death domain; TIR, Toll/Interleukin-1 receptor domain; CARD, caspase activation and recruitment domain; MyD88, myeloid differentiation primary response gene 88; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin; SRC, steroid receptor coactivator.

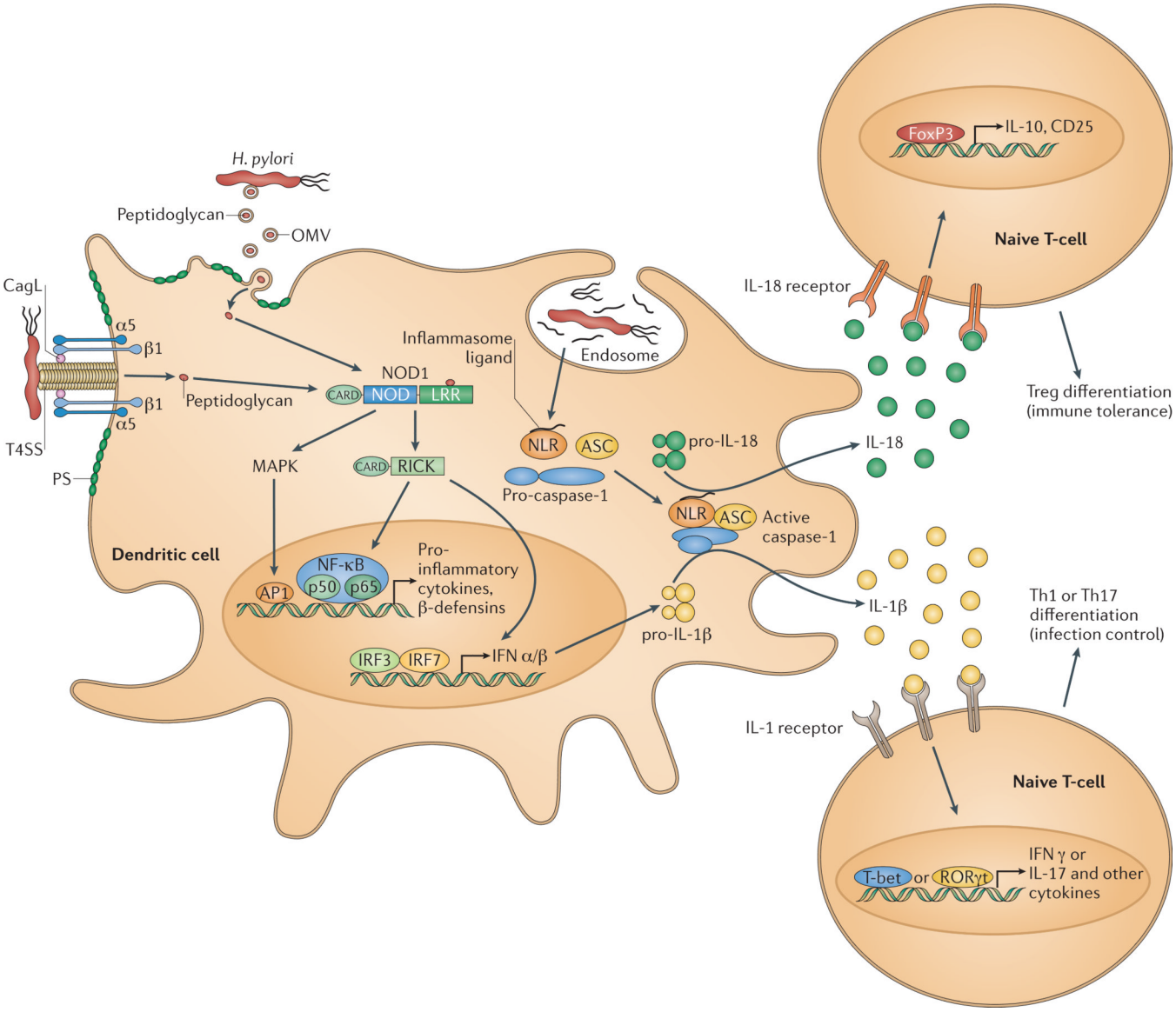


Figure 3. *H. pylori* activation of NLRs, NF-κB signalling and caspase-1

H. pylori peptidoglycan is delivered to the cytoplasmic NLR Nod1 through either the type IV secretion system (T4SS; via its interaction with α5β1 integrin at cholesterol-rich lipid rafts) or via outer membrane vesicles. Activated Nod1 induces the AP1/NF-κB-dependent expression of pro-inflammatory cytokines and defensins, and the IRF3/7-dependent expression of type I IFNs. Additional unidentified *H. pylori* NLR ligands activate the inflammasome to induce autoproteolytic pro-caspase-1 cleavage and the subsequent processing and release of mature IL-1β and IL-18. IL-18 binds to its receptor on naive T-cells and promotes FoxP3-dependent Treg differentiation and immune tolerance, which in turn prevents clearance and ensures persistent colonization of *H. pylori*. In contrast,IL-1β binding to its receptor induces T-bet- and RORγT-dependent Th1 and Th17 differentiation and the expression of the respective signature cytokines IFN-γ and IL-17. Please note that the pictured innate immune cell is a dendritic cell, whereas peptidoglycan-induced Nod1 signalling has been demonstrated in gastric epithelial cells. OMVs, outer membrane vesicles; ASC, apoptosis-associated speck-like protein containing a carboxy-terminal CARD

domain; NOD, nucleotide-binding oligomerization domain; LRR, leucine-rich repeat domain; RICK, receptor-interacting serine/threonine kinase; MAPK, MAP kinase.

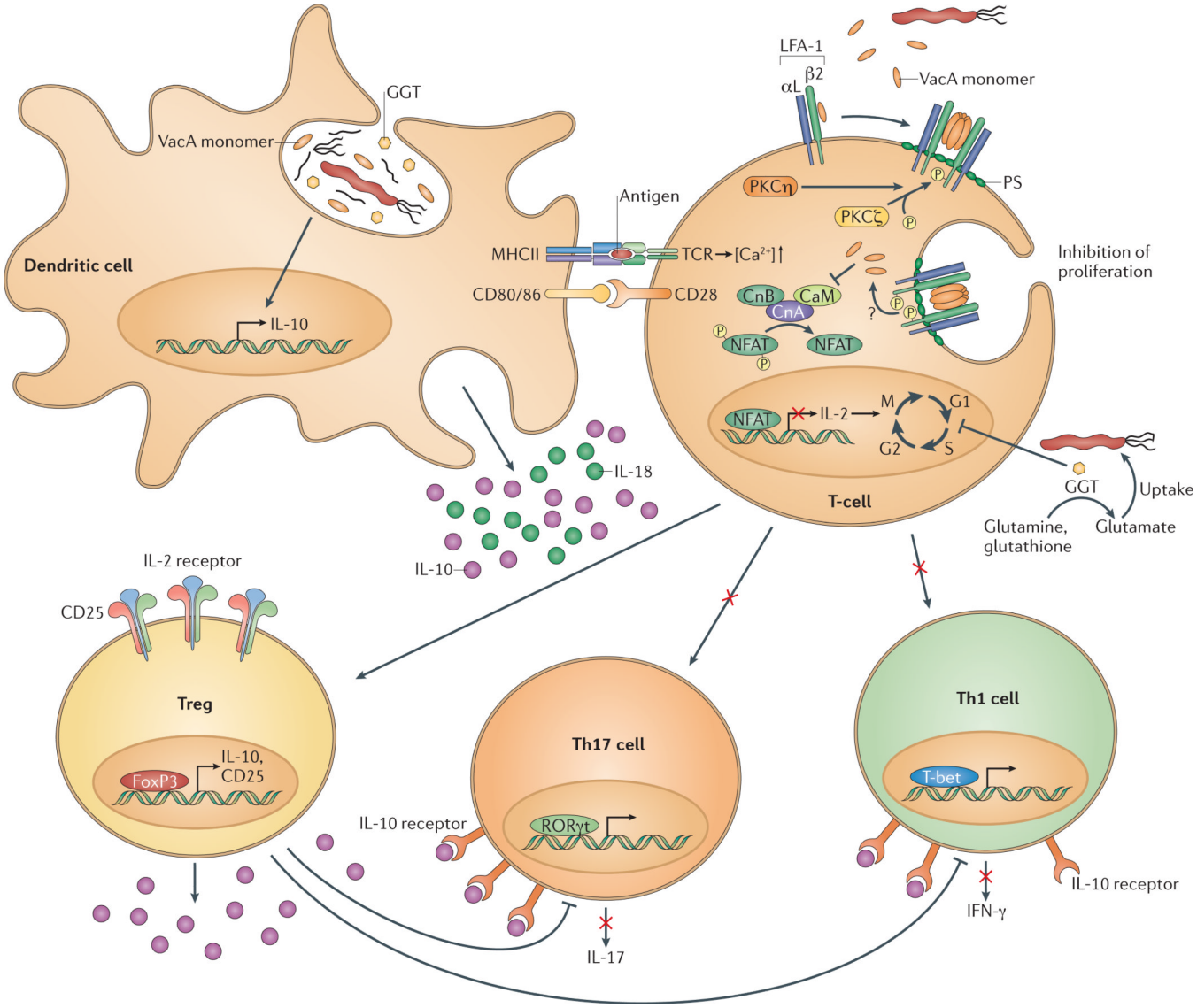


Figure 4. *H. pylori* impairs T-cell-mediated immunity via direct and indirect mechanisms

All strains of *H. pylori* express the secreted virulence factors VacA and GGT to directly inhibit T-cell activation, proliferation and effector functions. Hexameric VacA binds to the β2 integrin subunit of the heterodimeric transmembrane receptor LFA-1; the receptor complex is internalized upon protein kinase C-mediated serine/threonine phosphorylation of the β2 integrin cytoplasmic tail. Cytoplasmic VacA prevents nuclear translocation of NF-AT by inhibiting its dephosphorylation by the Ca^{2+}/calmodulin-dependent phosphatase calcineurin, and thereby blocks IL-2 production and subsequent T-cell activation and proliferation. GGT arrests T-cells in the G1 phase of the cell cycle and thus prevents their proliferation. Both VacA and GGT also indirectly prevent T-cell immunity via re-programming of DCs; VacA/GGT-exposed DCs produce IL-10, and induce the FoxP3- and contact-dependent differentiation of T-cells into regulatory T-cells while at the same time preventing Th1 and Th17 differentiation. Depicted interactions at the T-cell/DC synapse include MHCII binding to the T-cell receptor and binding of costimulatory molecules CD80/CD86 to CD28. DC-derived and/or Treg-derived IL-10 further suppresses Th1 and Th17 effector functions. Note that the direct effects of VacA on T-cells appear to be human-

specific, whereas indirect effects of VacA and GGT on T-cells via DCs have only been documented in the murine system. LFA-1, lymphocyte function-associated antigen-1; NF-AT- nuclear factor of activated T-cells; GGT, γ-glutamyl-transpeptidase; CnA,B, calcineurin A and B subunits; CaM, calmodulin; RORγT, retinoid-related orphan receptor γT; T-bet, T-box transcription factor.