Chapter 3
Virulence Mechanisms of *Helicobacter pylori*: An Overview

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Abstract *Helicobacter pylori* is a highly successful human pathogen, able to establish a chronic infection in the harsh environment of the stomach. These bacteria express a variety of virulence factors that promote their survival under acidic conditions, motility and spatial orientation in gastric mucus and adherence to epithelial cells. Other pathogenicity-associated mechanisms contribute to chronic gastritis by inducing pro-inflammatory responses and by manipulating cellular responses in host cells. Although *H. pylori* elicits a strong inflammatory response, the immune system fails to clear the infection. The pathogen employs a range of evasion strategies to dampen or reduce host immune responses. These strategies enable *H. pylori* to establish an equilibrium with its host, so that the vast majority of the chronically infected individuals do not develop severe disease. However, in a subset of patients, disturbance of this equilibrium in favour of the pathogen may lead to the development of gastroduodenal ulceration, mucosa-associated lymphoid tissue (MALT) lymphoma or adenocarcinoma.

Keywords Virulence factors • Pathogenesis • Urease • Motility • Adhesion • Immunomodulation • Apoptosis • Autophagy

3.1 Introduction

Helicobacter pylori is one of the most successful human pathogens, colonising more than 50 % of the world's population (Suerbaum and Josenhans 2007). The infection is usually acquired in early childhood (Weyermann et al. 2009) and, in the absence of aggressive antibiotic therapy, typically persists for life (Suerbaum and

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Josenhans 2007). Most of the colonised individuals develop asymptomatic chronic gastritis, but in 10–20 % of cases, *H. pylori* infection is associated with the development of severe gastroduodenal disease, including peptic ulcers, gastric MALT lymphoma or adenocarcinoma (Kusters et al. 2006). *H. pylori* infection is the strongest known risk factor for gastric adenocarcinoma, and in 1994 the International Agency for Research on Cancer classified *H. pylori* as a class I carcinogen. The clinical outcome of *H. pylori* infection depends on a complex interplay of many factors, including the virulence determinants expressed by the colonising strain(s), the genetic background of the host and environmental factors.

H. pylori strains show extensive genetic diversity, which is a consequence of the high mutation (Bjorkholm et al. 2001) and recombination frequencies (Suerbaum et al. 1998) in this bacterium. *H. pylori* has undergone a long co-evolution with its human host. Indeed, it is estimated that the association of *H. pylori* with modern humans predates by some 40,000 years the human migration from East Africa that occurred approximately 60,000 years ago (Moodley et al. 2012) (see Chap. 9 for more details). During this long association with humans, *H. pylori* has evolved sophisticated mechanisms to persistently colonise its host and avoid elimination by the immune system. These mechanisms range from colonisation factors that allow the bacterium to survive the harsh acidic environment of the stomach and establish persistent infection in the gastric mucosa, to complex strategies involving virulence factors that enable *H. pylori* to evade and manipulate both innate and adaptive immune responses. The lack of disease progression in the vast majority of persistently colonised individuals points to a delicate balance between the host and the pathogen. This chapter summarises the bacterial factors and biological processes that enable *H. pylori* to establish persistent colonisation and chronic inflammation of the human gastric mucosa. A more in-depth analysis of several of these products and processes will be provided in the chapters to follow.

3.2 *H. pylori* Colonisation and Adherence

3.2.1 Escape from the Stomach Lumen

In order to reach its ideal ecological niche, *H. pylori* must survive the extremely acidic environment of the stomach lumen and penetrate the outer mucous gel layer of the stomach. Once in the mucus, *H. pylori* resides in a very specific niche with an external pH of approximately 5–6. The bacterium is, however, able to increase the pH of its immediate surrounds, as well as of its cytosol and periplasm, by producing urease, which hydrolyses urea to ammonium ions and carbon dioxide (Marshall et al. 1990). Urease is composed of UreA and UreB subunits (Labigne et al. 1991) which are assembled into a catalytically active, nickel-containing dodecamer via the actions of accessory proteins UreE, UreF, UreG and UreH (Mobley et al. 1995). Urease activity is up-regulated under acidic conditions, by a proton-gated urea

channel formed by the inner membrane protein, UreI, allowing rapid entry of urea into the bacteria (Skouloubris et al. 1998; Weeks et al. 2000). *H. pylori* can further tightly control urease activity in response to both an acidic pH and increasing concentrations of nickel ions. This occurs via up-regulation of urease gene expression by the acid-responsive signalling regulon (ArsRS) (Pflock et al. 2006) and the nickel response regulator, NikR (van Vliet et al. 2002), respectively. *H. pylori* mutants lacking either urease activity (through disruption of *ureB*) or a functional UreI were shown to be defective for colonisation in animal models of infection (Eaton et al. 2002; Skouloubris et al. 1998), thus demonstrating the essential role of urease in *H. pylori* pathogenesis (see also Chap. 2).

Having overcome the acidic lumen, *H. pylori* must next confront the viscous mucous gel covering the gastric epithelium. Gastric mucus varies in its viscoelastic properties, from a soft gel at acidic pH to a viscous solution at neutral pH. *H. pylori* is well adapted to this environment and is able to move rapidly, in a corkscrew like manner, through highly viscous solutions that impede the motility of rod-shaped organisms, such as *Escherichia coli* (Hazell et al. 1986). The spiral cell shape of *H. pylori* is believed to enhance its ability to penetrate the mucus, and mutants lacking a helical twist show a colonisation defect. Production and maintenance of the spiral morphology require coordinated activity of multiple enzyme networks that modify the peptidoglycan composition of the cell wall (Bonis et al. 2010; Sycuro et al. 2012).

In addition to its spiral shape, *H. pylori* uses flagella-mediated motility to move through both the gastric lumen and mucus to reach and maintain itself close to the epithelial surface. The importance of motility for *H. pylori* is illustrated by the fact that mutants lacking flagella were unable to colonise the gnotobiotic piglet model of infection (Eaton et al. 1996). *H. pylori* bacteria have two to five sheathed, unipolar flagella which are composed of the basal body, the hook/universal joint and the filament. The flagellar filament is composed of repeating units of two flagellins, FlaA (53 kilodalton, kDa) and FlaB (54 kDa) (Suerbaum et al. 1993). The *flaA* and *flaB* genes are not co-located on the chromosome, nor is their transcription co-regulated (Suerbaum et al. 1993). The flagellar system comprises a network of over 40 mostly unclustered and temporally regulated genes, whose transcription is hierarchical and tightly controlled by the three RNA polymerase sigma factors of *H. pylori*: σ^{28} (FliA), σ^{54} (RpoN) and σ^{80} (Douillard et al. 2009; Josenhans et al. 2002; McGowan et al. 2003; Niehus et al. 2004). *H. pylori* also has an anti-sigma factor, FlgM, which acts as an antagonist to FlaA (Colland et al. 2001; Niehus et al. 2004). Flagellar assembly requires interaction with the peptidoglycan layer through which the flagella has to be extruded. The peptidoglycan-degrading enzymes of the lytic transglycosylase family, Slt and MltD, are required for full motility in *H. pylori* (Roure et al. 2012). Inactivation of *mltD*, but not *slt*, was shown to have a significant impact on *H. pylori* colonisation in vivo (Roure et al. 2012).

Motile bacteria use chemotaxis for spatial orientation, coupling control of flagellar rotation with environmental sensing (Wadhams and Armitage 2004). *H. pylori* uses four methyl-accepting chemoreceptor proteins (TlpA, TlpB, TlpC and TlpD) to sense the external stimuli and repellent ligands. This information is

then relayed via CheW, to a histidine kinase, CheA, which phosphorylates the response regulator, CheY. The phosphorylated CheY interacts with the flagellar motor to alter the rotational direction of the flagellum. *H. pylori* mutants lacking *cheA*, *cheW* or *cheY* are non-chemotactic and show colonisation defects (Terry et al. 2005). In addition, *H. pylori* encodes a novel chemotaxis regulator, ChePep, which preferentially localises to the flagellar pole. *H. pylori chePep* mutants cannot control the rotation of their flagella, but are motile. They are attenuated for colonisation of the stomach and fail to establish bacterial colonies deep in the gastric glands of mice. Interestingly, ChePep homologues are present and functionally conserved in ε-proteobacteria, but not in other bacterial classes (Howitt et al. 2011).

Microarray analyses of *H. pylori* showed that both exposure to low pH in vitro and infection of the gerbil stomach in vivo resulted in increased expression of many of the genes involved in motility and chemotaxis (Merrell et al. 2003; Scott et al. 2007). These findings are consistent with data showing that exposure of *H. pylori* to an acidic environment leads to a large increase in both the proportion and the speed of motile bacteria (Merrell et al. 2003). *H. pylori* exploits the pH gradient of the stomach, which ranges from pH 1.8 in the lumen to a near-neutral pH at the mucus-mucosal interface, to guide it to the epithelial surface. This pH-tactic behaviour is dependent on the chemotaxis receptor, TlpB (Croxen et al. 2006). *H. pylori tlpB* mutants were shown to be motile but could not colonise interleukin-12 p40 (IL-12 p40)-deficient C57BL/6 mice (Croxen et al. 2006). Expression of *tlpB* is regulated at the posttranscriptional level by an abundant small RNA (sRNA), regulator of polymeric G-repeats (RepG), which targets a homopolymeric G-repeat in the leader region of the *tlpB* mRNA. The length of this G-repeat, which varies from 6 to 16 guanine residues in different *H. pylori* strains, influences both the level and type (repression or activation) of regulation. There is also evidence that the length of the *tlpB* G-repeat can change during infection, suggesting that differential expression of *tlpB* may be involved in host adaptation (Pernitzsch et al. 2014).

3.2.2 *Adhesion of* H. pylori *to Gastric Epithelial Cells*

Although most of the *H. pylori* in the mucosa are free-swimming, some 20 % of the bacteria adhere to the surface of the epithelial cells (Hessey et al. 1990). Binding of *H. pylori* to gastric epithelial cells involves the interactions between specific bacterial adhesins and their cognate receptors on the surfaces of host cells. The known *H. pylori* adhesins all belong to the major outer membrane protein (OMP) family 1 (Alm et al. 2000). This family of proteins is further divided into the *Helicobacter* outer membrane porins (Hop) and Hop-related (Hor) subgroups (Alm et al. 2000; Odenbreit et al. 2009). All but one of the adhesins identified to date are members of the Hop family. The best characterised of these adhesins are the blood group antigen-binding (BabA) and sialic acid-binding (SabA) proteins,

the outer inflammatory protein A (OipA) and the adherence-associated lipoproteins, AlpA and AlpB.

BabA binds to the human fucosylated Lewis^b antigen (Le^b) and related terminal fucose residues on blood group O (H antigen), A and B antigens present on the surface of gastric epithelial cells (Aspholm-Hurtig et al. 2004; Gerhard et al. 1999; Ilver et al. 1998). BabA binding affinity for O, A and B antigens correlates with the blood group expressed by the human host, supporting the notion of adaptation to the host during persistent infection and transmission between hosts (Aspholm-Hurtig et al. 2004). In contrast, SabA binds to sialyl-Lewis x (sLe^x) and Lewis a (sLe^a) antigens whose synthesis is up-regulated during *H. pylori*-induced inflammation (Mahdavi et al. 2002). SabA also binds to erythrocytes (Aspholm et al. 2006) and may play a role in inflammation, by binding and activating neutrophils (Unemo et al. 2005). More details on the effects of BabA and SabA on gastroduodenal disease can be found in Chap. 6.

OipA (formerly called HopH) is involved in the adherence of *H. pylori* to gastric cell lines (Dossumbekova et al. 2006; Yamaoka et al. 2004), but its host receptor has yet to be identified. Expression of functional OipA, which is regulated by slipped-strand mispairing within a CT-rich region present at the 5′ terminus of *oipA*, promoted IL-8 induction in vitro. Conversely, *oipA* inactivation in *cag*PAI$^+$ clinical isolates resulted in approximately 50 % lower IL-8 responses in epithelial cells (Yamaoka et al. 2004). The notion that OipA has a role in IL-8 production in vivo is supported by the observation that OipA expression was significantly associated with high levels of IL-8 in the gastric mucosa of infected patients (Yamaoka et al. 2002). The molecular basis for the effect of OipA on IL-8 production was investigated in a study of the IL-8 promoter in gastric cell cultures (Yamaoka et al. 2004). This study showed that maximal induction of IL-8 transcription required activation of an interferon-stimulated responsive element (ISRE)-like element by the interferon regulatory factor (IRF)-1 (Yamaoka et al. 2004). Moreover, OipA was reported to selectively induce phosphorylation of signal transducer and activator of transcription 1 (STAT1), an upstream mediator of IRF-1 signalling. These results were recapitulated in vivo, where it was found that STAT1 phosphorylation in human gastric biopsy specimens correlated with the presence of functional OipA in the infecting *H. pylori* strain (Yamaoka et al. 2004). It has also been proposed that OipA plays a role in IL-1β, IL-17 and TNF expression and inflammation in the stomach (Sugimoto et al. 2009). Analysis of clinical isolates showed that the presence of functional OipA is associated with high *H. pylori* density, severe neutrophil infiltration, duodenal ulcer disease and gastric cancer (Yamaoka et al. 2002, 2006; Franco et al. 2008). The role of OipA in gastric disease is supported by data showing that inactivation of *oipA* reduces the incidence of cancer in Mongolian gerbils and decreases nuclear translocation of β-catenin (Franco et al. 2008), a cellular protein important for cell adhesion and regulation of genes implicated in carcinogenesis. In vitro studies with murine dendritic cells (DCs) showed that OipA suppresses DC maturation and decreases production of IL-10 (Teymournejad et al. 2014); however, the biological significance of this finding requires further investigation.

AlpA and AlpB, which are encoded by the *alpA/B* operon, are also required for specific adhesion of *H. pylori* to human gastric epithelial cells (Odenbreit et al. 1999). Furthermore, these proteins were shown to be important for colonisation in guinea pig (de Jonge et al. 2004) and murine (Lu et al. 2007) models of infection. Analysis of 200 clinical strains showed that AlpA and AlpB were expressed in all strains, suggesting that these adhesins are likely to have important functions (Odenbreit et al. 2009). The target of both AlpA and AlpB is laminin, a component of the host extracellular matrix (Senkovich et al. 2011).

3.3 Major *H. pylori* Virulence Factors Involved in Pathogenesis

3.3.1 cag *Pathogenicity Island (*cag*PAI)*

The *cag*PAI is a horizontally acquired insertion element of 40-kilobases (kb), consisting of approximately 31 genes, whose presence in a functional form is associated with an increased risk of severe gastroduodenal disease (Covacci et al. 1999; see also Chap. 4). *cag*PAI encodes a bacterial type IV secretion system (T4SS) and its only known effector protein, CagA, which translocates into gastric epithelial cells (Censini et al. 1996; Fischer et al. 2001; Odenbreit et al. 2000). The presence of a *cag*PAI appears to influence the topography of colonisation within the stomach, as *cag*PAI$^-$ *H. pylori* strains were mostly present in the mucous gel layer or near the apical surface of epithelial cells, whereas the *cag*PAI$^+$ strains were found closely adjacent to gastric epithelial cells or in the intercellular epithelial spaces (Camorlinga-Ponce et al. 2004).

The *H. pylori* T4SS is induced by contact with the host cell and forms a large complex spanning the inner and outer membranes of the bacterium, with a pilus-like structure that protrudes from the bacterial surface (Rohde et al. 2003). It is currently unclear how the *H. pylori* T4SS is able to deliver not only CagA but also *H. pylori* cell wall peptidoglycan, into the host cell. The internalised peptidoglycan is recognised by the cytoplasmic pathogen-recognition molecule, nucleotide-binding oligomerisation domain-containing protein 1 (NOD1) (Viala et al. 2004). NOD1 sensing of *H. pylori* peptidoglycan triggers in epithelial cells a pro-inflammatory signalling cascade, characterised by the translocation of nuclear factor-κB (NF-κB) to the nucleus (Viala et al. 2004) and activation of the mitogen-activated protein kinases (MAPKs), p38 and extracellular signal-regulated kinase (ERK), leading to induction of CXC chemokine responses (Allison et al. 2009). *H. pylori* can also activate this pro-inflammatory signalling cascade via the actions of outer membrane vesicles (OMVs) which deliver peptidoglycan to cytosolic NOD1 (Hutton et al. 2010; Kaparakis et al. 2010). Both T4SS- and OMV-dependent activations of the NOD1 signalling pathway involve cholesterol-rich microdomains, or lipid rafts, in host-cell membranes (Hutton et al. 2010; Kaparakis

et al. 2010). Interestingly, CagA translocation into host cells also requires the presence of functional lipid raft domains (Jimenez-Soto et al. 2009; Lai et al. 2008). *H. pylori* T4SS delivery of CagA (Jimenez-Soto et al. 2009; Kwok et al. 2007) and peptidoglycan (Hutton et al. 2010; Kaparakis et al. 2010) into cells was shown to be dependent upon binding of the *cag*PAI-encoded protein, CagL, to its cognate host-cell receptor, $\alpha_5\beta_1$ integrin. CagA itself was shown to interact with the host factors, β_1 integrin (Jimenez-Soto et al. 2009; Kwok et al. 2007) and phosphatidylserine (Murata-Kamiya et al. 2010).

Once CagA has translocated into epithelial cells, it localises to the plasma membrane and undergoes tyrosine phosphorylation within the EPIYA motif that is found in tandemly arranged segments located in the C-terminal half of the protein. The number and organisation of these segments differ between *H. pylori* strains and are thought to contribute to differences in strain pathogenicity (Argent et al. 2004; Higashi et al. 2002). There are four distinct EPIYA segments (A to D), each of which contains a single EPIYA motif, with the EPIYA A, B and C segments predominating in *H. pylori* isolates from Western countries and EPIYA A, B and D segments predominating in the generally more virulent East Asian isolates (Higashi et al. 2002). The cellular kinases responsible for phosphorylating the EPIYA motifs within CagA are oncoproteins belonging to the Src and Abl family kinases (Poppe et al. 2007; Selbach et al. 2002; Tammer et al. 2007).

CagA translocation and tyrosine phosphorylation lead to a perturbation of mammalian signal transduction cascades, morphological effects such as cell cytoskeletal rearrangement, elongation and scattering that has been designated the "hummingbird" phenotype, as well as modification of cellular functions (Selbach et al. 2003; Tammer et al. 2007; Tsutsumi et al. 2003). These in vitro observations are recapitulated in vivo, with the finding that CagA is actively translocated to gastric epithelial cells and tyrosine-phosphorylated and binds Src homology region 2 (SH2) domain-containing phosphatase-2 (SHP-2) in inflamed human gastric mucosa (Tsutsumi et al. 2003). The ability of CagA to perturb host-cell functions is dependent on its SHP-2 binding activity, which is determined by the number and sequences of tyrosine phosphorylation sites (Higashi et al. 2002). It should be noted that non-phosphorylated CagA also contributes to pathogenesis, through interactions that lead to induction of pro-inflammatory and mitogenic responses, suppression of apoptosis, loss of cell polarity and disruption of gastric barrier function (see Chap. 4 for a detailed discussion).

3.3.2 Vacuolating Cytotoxin VacA

VacA is a pore-forming toxin secreted by a classical autotransporter pathway (see Chap. 5 for more details). The protein is synthesised as a 140 kDa precursor, which is processed to produce a 33-amino acid signal peptide, the mature 88 kDa secreted toxin, an approximately 12 kDa secreted peptide and a C-terminal domain that remains associated with the bacteria (Cover and Blaser 1992; Schmitt and Haas

1994; Telford et al. 1994). The secreted VacA can undergo spontaneous proteolytic cleavage into the N-terminal p33 and C-terminal p55 fragments that are thought to represent the functional domains of VacA (Torres et al. 2005) and that remain non-covalently associated (Cover et al. 1997; Lupetti et al. 1996; Telford et al. 1994). The p33 domain is important for membrane channel formation (McClain et al. 2001; Ye et al. 1999), whereas the p55 domain is required for binding to host cells (Garner and Cover 1996). Both domains are required for toxin oligomerisation (Gangwer et al. 2007; Genisset et al. 2006). Active VacA induces structural and functional changes in epithelial cells in vitro, the most noticeable being formation of large intracellular vacuoles, the phenotype that gave the toxin its name (Leunk et al. 1988).

Most of the secreted VacA was shown to bind to cultured epithelial cells and to use lipid rafts as entry sites so as to be internalised by clathrin-independent endocytosis (Gauthier et al. 2005). A number of studies have indicated that VacA may also exert an antagonistic effect on CagA functions (see Chap. 5 for a detailed discussion). Once intracellular, VacA causes a wide range of alterations to the host cell. The large membrane-bound vacuoles induced by VacA in the cytoplasm of gastric cells originate from the late endosomal pathway and are a consequence of disruption of the late endosomal/lysosomal compartments (Papini et al. 1994). However, the role of these cytoplasmic vacuoles in *H. pylori* pathogenesis is unclear. As discussed in Sect. 6 below, VacA induces apoptosis of gastric epithelial cells, independently of its vacuolating activity and also promotes autophagy in these cells.

All *H. pylori* strains encode *vacA*, yet display considerable heterogeneity in their production of the vacuolating cell phenotype (Atherton et al. 1995). This diversity is largely due to polymorphisms in the *vacA* gene. The highest level of sequence diversity is found in the signal (s), intermediate (i) and middle (m) regions of *vacA* and forms the basis of a classification system. The signal sequences of the s1 and s2 alleles of VacA are processed at different sites, such that the mature s2 toxin contains a 12-amino acid hydrophilic extension at its N-terminus, which abolishes its cytotoxic activity and reduces its ability to form membrane channels, without abrogating toxin secretion (Letley et al. 2003; McClain et al. 2001). The i region, defined as either i1 or i2, is important for toxicity (Rhead et al. 2007; Winter et al. 2014); however, its role in VacA functions is not yet known. The m region of VacA contains the cell-binding site, with m1-type toxins having higher binding affinities for host cells than m2-type toxins and also showing different cell-type specificities (Pagliaccia et al. 1998; Wang et al. 2001).

H. pylori strains with the s1/m1 *vacA* alleles have higher levels of vacuolating activity in vitro than those carrying s1/m2 alleles (Atherton et al. 1995). Epidemiological studies are consistent with these in vitro observations, as *H. pylori* strains that encode s1 and m1 *vacA* alleles are associated with a higher risk of gastric carcinoma than strains with s2 and m2 alleles. Furthermore, s1/m1 *vacA* genotypes are strongly associated with peptic ulcers (Atherton et al. 1995, 1997; Strobel et al. 1998). The i1 allele of *vacA* shows a strong association with gastric adenocarcinoma (Rhead et al. 2007; Winter et al. 2014). Interestingly, in the murine

model of infection, *H. pylori* bacteria producing the s2/i2 form of VacA colonised mice more efficiently than those producing the s1/i1 form of VacA or those lacking VacA, potentially suggesting a different biological role for the weakly active s2/i2 toxin. Strains producing more active VacA induced more severe and extensive metaplasia and inflammation in the mouse stomach than strains producing s2/i2 toxin (Winter et al. 2014). Thus, specific *vacA* alleles may contribute to the pathogenicity and clinical outcomes of *H. pylori* infection.

3.3.3 *Other Putative Autotransporter Proteins of* H. pylori

H. pylori genomes contain three *vacA*-like genes encoding proteins of 260–348 kDa (Tomb et al. 1997). The C-terminal regions of these proteins show homology to the C-terminal region of VacA, which is a β-barrel domain that is required for secretion of VacA through an autotransporter (type V) pathway. On the basis of this similarity, three proteins are predicted to be autotransporters: immunomodulating autotransporter (ImaA), flagella-associated autotransporter A (FaaA) and VacA-like protein C (VlpC) (Radin et al. 2013; Sause et al. 2012). These proteins are all present on the surface of *H. pylori* (Radin et al. 2013; Sause et al. 2012). However, whereas ImaA and VlpC localise to a bacterial pole, FaaA localises to a sheath overlying the flagellar filament and bulb and is important for flagellar morphology and function. The *faaA* mutant strain shows decreased motility, reduced flagellar stability and an increased proportion of flagella in nonpolar sites (Radin et al. 2013).

Expression levels of *imaA*, *faaA* and *vlpC* were up-regulated during colonisation of the mouse stomach (Radin et al. 2013; Sause et al. 2012). *imaA* was identified as belonging to the ArsRS regulon and thus its increased expression in vivo is likely a response to gastric acid (Sause et al. 2012). The mechanism(s) by which *faaA* and *vlpC* expression levels are regulated remain(s) unknown. Consistent with the idea that ImaA, FaaA and VlpC may have important roles in colonisation, competition experiments in mice showed that mutants for each of these autotransporters were outcompeted by wild-type bacteria in vivo (Radin et al. 2013; Sause et al. 2012). Indeed, a single challenge study confirmed that an *H. pylori faaA* mutant was attenuated in its ability to colonise, when compared with the wild-type strain; however, this was apparent during the early (4 days post-infection) but not late (1 month post-infection) stages of infection (Radin et al. 2013; Sause et al. 2012). It was suggested that FaaA may be important early in the infection process, when fully formed and functional flagella are required for *H. pylori* entry into the mucous layer (Radin et al. 2013; Sause et al. 2012). Similar to the findings above, mice challenged with *H. pylori imaA* mutant bacteria alone or in competition with wild-type bacteria demonstrated that this mutant also had a colonisation defect in vivo (Sause et al. 2012). In this case, however, ImaA reduced expression of inflammatory chemokines and cytokines in infected stomachs and cultured epithelial cells, suggesting that this autrotransporter may be important for dampening host immune responses (Sause et al. 2012). The immunomodulatory activity of ImaA was

observed in *H. pylori* strains that harbour a *cag*PAI, suggesting that ImaA down-regulates the inflammatory responses triggered by the T4SS (Sause et al. 2012). Interestingly, ImaA exhibits some similarity to the bacterial integrin-binding protein, invasin (Sause et al. 2012). As the T4SS pilus is known to mediate its pro-inflammatory effects through binding to $\alpha_5\beta_1$ integrin (Hutton et al. 2010; Kwok et al. 2007), it was suggested that ImaA and the T4SS may compete for integrin binding (Sause et al. 2012). Thus, in the absence of ImaA, the T4SS is better able to deliver the pro-inflammatory effectors, CagA and peptidoglycan (Sause et al. 2012).

3.3.4 γ-Glutamyl Transpeptidase

γ-Glutamyl transpeptidase (GGT) is produced by all strains of *H. pylori* (Chevalier et al. 1999). It plays an important role in the amino acid metabolism of *H. pylori*, by synthesising glutamate from both glutamine and glutathione, neither of which can be assimilated from the environment by this bacterium (Shibayama et al. 2007). *H. pylori* GGT hydrolyses glutamine and glutathione outside the cell, with the resulting products of this reaction being glutamate and ammonia and glutamate and cysteinylglycine, respectively. The glutamate produced is transported by a Na^+-dependent reaction into *H. pylori* cells (Shibayama et al. 2007). The enzyme is catalytically active even at the low pH of the gastric mucosa and its expression appears to be constitutive (Chevalier et al. 1999). GGT is synthesised as a 60 kDa inactive proenzyme that undergoes autocatalytic processing to form an enzymatically active heterodimer of 40 and 20 kDa subunits (Chevalier et al. 1999; Shibayama et al. 2003).

Several lines of evidence indicate that GGT is a virulence factor of *H. pylori*. Although deletion of *ggt* did not impair the in vitro growth of *H. pylori* (Chevalier et al. 1999), *ggt* mutants were attenuated for colonisation of mice and gnotobiotic piglets (Chevalier et al. 1999; McGovern et al. 2001; Oertli et al. 2013). The degree of attenuation appears to depend on the *H. pylori* strain and/or the experimental animals used. It has been suggested that GGT may be associated with *H. pylori*-induced peptic ulcer disease (PUD) in that *H. pylori* isolates from patients with PUD showed significantly higher levels of GGT activity than those from patients with non-ulcer dyspepsia (Gong et al. 2010). Definitive evidence for this suggestion is, however, currently lacking.

GGT is thought to contribute to the *H. pylori*-induced damage to the gastric epithelial cells by promoting apoptosis and by modulating the immune response. Purified, enzymatically active GGT induced apoptosis and reduced viability in AGS gastric epithelial cells (Shibayama et al. 2003). Furthermore, GGT was shown to induce production of H_2O_2, leading to DNA damage, apoptosis and activation of inflammatory pathways (Gong et al. 2010). It has been suggested that GGT might contribute to the damaging effect of *H. pylori* on gastric cells by depleting glutamine and glutathione, which are important nutrients for maintenance of healthy

gastrointestinal tissue (Shibayama et al. 2007). As discussed in Sect. 4.3 and in more detail in Chap. 5, GGT also exerts immunomodulatory effects on T-cells.

3.3.5 *High Temperature Requirement A (HtrA) Serine Protease*

H. pylori HtrA belongs to a family of serine proteases that is widely conserved in both single and multicellular organisms. This family of proteins can be distinguished from other serine proteases by their sequence homology and oligomeric structure, as well as by the presence of a protease domain and one or two carboxy terminal PDZ (**p**ost synaptic density protein, **D**rosophila disc large tumour suppressor, Dlg1, and **z**onula occludens-1 protein) domains (Clausen et al. 2011). HtrA serine proteases are involved in important cellular processes, including bacterial virulence (Clausen et al. 2011). The HtrA secreted by *H. pylori* (Bumann et al. 2002) cleaves the extracellular domain of the cell adhesion protein E-cadherin, present on the surface of host cells, resulting in the loss of cell-cell contact and enabling the bacterial entry into the intracellular space of epithelial cells (Hoy et al. 2010). *H. pylori* HtrA cleavage of E-cadherin was highly efficient at physiological and high temperatures and at pH 5–8, with highest activity observed at pH 6–7 (Hoy et al. 2013). Expression of HtrA was up-regulated by oxidative stress (Huang and Chiou 2011) and environmental acidification (Merrell et al. 2003). These characteristics of HtrA might aid *H. pylori* in colonising the gastric environment. Interestingly, HtrA also appears to be essential for *H. pylori* survival in vitro (Hoy et al. 2010; Salama et al. 2004), suggesting that the protein may have functions other than the cleavage of E-cadherin. Indeed, many HtrAs play an important role in protein quality control, with some also acting as chaperones to stabilise specific proteins (Clausen et al. 2011).

3.3.6 *Other Pro-inflammatory Virulence Factors of* H. pylori

Various new bacterial virulence factors are emerging as putative contributors to *H. pylori* pathogenesis in human gastric mucosa. For example, it has been suggested that the TNFα-inducing protein (Tipα, HP0596) contributes to *H. pylori* oncogenicity. Tipα is a homodimeric protein that has been shown to be important for colonisation of mouse gastric mucosa (Godlewska et al. 2008) and is secreted independently of the T4SS (Suganuma et al. 2005). This protein binds specifically to nucleolin, a cell surface receptor on gastric epithelial cells (Watanabe et al. 2010), whereupon it is internalised into the cytosol and then nucleus (Suganuma et al. 2008). In a mouse gastric epithelial cell line (MGT-40), Tipα was shown to induce expression of chemokine genes, such as *Ccl2*, *Ccl7*, *Ccl20*,

Cxcl1, *Cxcl2*, *Cxcl5* and *Cxcl10* (Kuzuhara et al. 2007). Tipα was also shown to induce epithelial-mesenchymal transition in human gastric cancer cell lines (Watanabe et al. 2014).

Duodenal ulcer-promoting gene A (*dupA*) was originally found to be associated with an increased risk for duodenal ulcers and a reduced risk for gastric atrophy and cancer (Lu et al. 2005). Subsequent studies, however, indicated that this association held in some geographical regions, but not in others (Abadi et al. 2012; Alam et al. 2012; Arachchi et al. 2007; Argent et al. 2007; Gomes et al. 2008; Imagawa et al. 2010; Nguyen et al. 2010; Shiota et al. 2010). *dupA* is associated with increased IL-8 production from gastric mucosa in vivo (Hussein et al. 2010; Lu et al. 2005). This gene is located in a plasticity region and encodes a protein that is functionally homologous to the T4SS ATPase protein, VirB4. In some *H. pylori* genomes, *dupA* is located adjacent to homologues of other *vir* genes, and a complete *dupA* cluster was predicted to form a third T4SS, *tfs3a* (Kersulyte et al. 2009). The presence of a complete *dupA* cluster was found to significantly increase the risk of duodenal ulcer compared to *H. pylori* infection with an incomplete *dupA* cluster or without the *dupA* gene (Jung et al. 2012). These data suggest that the epidemiological studies into the role of *dupA* in pathogenicity should be revisited, with the focus on the presence of an intact *dupA* cluster. Indeed, the extensive genetic diversity of *H. pylori*, which contributes to its success as a pathogen, also increases the difficulty of delineating the molecular basis of the pathogenesis of *H. pylori*-induced diseases.

3.4 Avoidance and Modulation of the Host Immune Response

Although *H. pylori* is an extracellular pathogen, this bacterium is able to disrupt epithelial integrity (Amieva et al. 2003). Thus, *H. pylori* products are likely to enter the lamina propria and come into contact with immune cells (Necchi et al. 2007). Although controversial, there is also some evidence to suggest that the bacterium can invade and replicate within intracellular compartments of epithelial cells, macrophages and DCs (Ricci et al. 2011). *H. pylori* induces vigorous inflammatory host responses, with a large influx of neutrophils, macrophages, DCs and lymphocytes, but the immune system fails to clear the infection, attesting to the success of the various sophisticated strategies used by the pathogen to evade and subvert this system. These strategies include: evasion of the innate immune system, modulation of phagocytosis and neutrophil functions, inhibition of lymphocyte proliferation, and skewing of T-cell-mediated adaptive immune responses toward tolerogenicity (Baldari et al. 2005).

3.4.1 Evasion of Detection by the Innate Immune System

Host cells are able to detect conserved components of microorganisms, known as microbe-associated molecular patterns (MAMPs), via pattern recognition receptors (PRRs). The best characterised PRRs are the Toll-like receptors (TLRs), which recognise specific classes of MAMPs and respond by activating intracellular signalling pathways that lead to activation of the master transcriptional regulator, NF-κB, and pro-inflammatory gene expression. *H. pylori* has developed several strategies that largely allow it to avoid detection by TLRs (Takeda and Akira 2005). The best understood of these strategies involve lipopolysaccharide (LPS) and flagellin.

LPS consists of an O side chain, a core oligosaccharide and lipid A, which is anchored to the bacterial membrane. The lipid A of *H. pylori* LPS is predominantly tetraacylated, whereas the LPS of *E. coli*, which is 1,000-fold more biologically active than that of *H. pylori*, is hexaacylated (Moran et al. 1997). Pathogenic bacteria can evade the host innate immune system by concealing or removing the negatively charged phosphate groups present on the lipid A disaccharide backbone. The resultant net loss of negative surface charges makes the bacterial membrane more resistant to cathelicidin antimicrobial peptides (CAMPs). CAMPs, which are found in macrophages and neutrophils and at the mucosal surface, are an important component of the host innate immune response and a link between the innate and adaptive immune systems (Diamond et al. 2009). The low biological activity of *H. pylori* LPS has been shown to be due to the removal of phosphate groups from the 1′- and 4′-positions of lipid A by two lipid phosphatases, LpxE and LpxF, respectively (Cullen et al. 2011). Dephosphorylated LPS is attenuated for TLR4 activation and highly resistant to CAMPs. Importantly, dephosphorylation of lipid A by LpxE and LpxF is required for effective colonisation and survival of *H. pylori* in mice (Cullen et al. 2011). Studies indicated that *H. pylori* LPS initiates inflammatory signalling in human epithelial cells via TLR2, rather than the more classical sensor of Gram-negative LPS, TLR4 (Smith et al. 2011; Yokota et al. 2007). TLR2 recognises a variety of microbial components, including lipoproteins, lipoteichoic acid and atypical LPS molecules whose structures differ from those recognised by TLR4 in the number of acyl chains in the lipid A moiety (Takeda and Akira 2005).

Recognition of many but not all bacterial flagellins by TLR5, which is present on the membrane of various cell types, including epithelial cells, leads to activation of the innate immune system (Takeda and Akira 2005). However, the major flagellin of *H. pylori*, FlaA, is much less well recognised by TLR5 than the flagellins of other enteric mucosal pathogens, such as *Salmonella typhimurium* (Gewirtz et al. 2004). Moreover, unlike the flagellins of *Escherichia* and *Salmonella*, FlaA is not released from the bacteria. The evolutionarily conserved recognition sequence for TLR5 is located in the N-terminal D1 domain of bacterial flagellin, within a region that is required for flagellar filament assembly and motility. Substitution of amino acids 89–96 of the flagellin (FliC) from *S. typhimurium*, with the corresponding amino acids from *H. pylori* FlaA, abolishes its recognition by TLR5 but also renders the bacteria non-motile (Andersen-Nissen et al. 2005). *H. pylori* has preserved its

motility by selecting for compensatory changes in other regions of FlaA, suggesting that avoidance of detection by TLR5 is important for the persistence of *H. pylori* at mucosal sites (Andersen-Nissen et al. 2005).

3.4.2 Modulation of Phagocytosis and Neutrophil Function

The engulfment and killing of microorganisms by the process known as phagocytosis are an important part of host innate defence against many pathogens. The role of macrophages in *H. pylori* pathogenesis, however, remains very controversial. Indeed, professional phagocytes appear to be ineffective in killing *H. pylori*. Reduced levels of *H. pylori* opsonisation by phagocytes have been attributed to its urease activity (Rokita et al. 1998) and the environmental conditions in the stomach (Berstad et al. 1997). Furthermore, *cag*PAI$^+$ *H. pylori* strains are able to retard phagocytosis in a *cag*PAI-dependent but CagA-, VacA- and urease-independent manner (Allen et al. 2000; Ramarao et al. 2000). Following their engulfment, these more virulent *H. pylori* strains stimulate rapid and extensive homotypic phagosome fusion, leading to formation of megasomes containing large numbers of viable *H. pylori*. Formation of these megasomes, which were shown to be stable for 24 h, requires live, metabolically active *H. pylori* (Allen et al. 2000). *cag*PAI$^+$ *H. pylori* strains induce the recruitment and retention of coronin 1 protein on phagosomes and prevent phagosome fusion with lysosomes (Zheng and Jones 2003). This inhibition of phagosome maturation is dependent on VacA and urease (Schwartz and Allen 2006; Zheng and Jones 2003). Although *H. pylori* strains that do not encode *cag*PAI, and which express a non-toxigenic form of VacA, are capable of subverting bacterial killing by macrophages for up to 24 h, their survival is inferior to that shown by *cag*PAI$^+$ bacteria (Zheng and Jones 2003). The ability of toxigenic alleles of VacA to modulate autophagy may also contribute to the survival of *H. pylori* in macrophages, by allowing the surviving phagocytosed bacteria to escape killing (Raju et al. 2012). Despite the in vitro evidence for *H. pylori* survival in macrophages, further investigations are required in in vivo models to confirm the biological relevance of these observations.

One mechanism by which *H. pylori* has been shown to be capable of interfering with its phagocytosis by antigen-presenting cells is via the actions of a cholesterol-**α**-glucosyltransferase (HP0421). This enzyme, also known as type 1 capsular polysaccharide biosynthesis protein J (CapJ), catalyses the conversion of cholesterol to cholesteryl α-glucosides (Lebrun et al. 2006; Wunder et al. 2006). Although *H. pylori* is auxotrophic for cholesterol, its envelope contains high concentrations of cholesteryl glucosides (Tannaes and Bukholm 2005). The pathogen extracts cholesterol from the plasma membranes of epithelial cells, but excessive cholesterol promotes phagocytosis of the bacteria by antigen-presenting cells, thereby enhancing T-cell activation. Conversely, α-glucosylation of cholesterol by cholesterol-α-glucosyltransferase abrogates phagocytosis of *H. pylori* and T-cell activation (Wunder et al. 2006). In addition to these effects, cholesterol

glucosylation by CapJ is important for tight binding of *H pylori* to gastric epithelial cells and for the assembly of a functional T4SS, as a *capJ* mutant was impaired in its ability to translocate CagA into the cytosol of host cells (Wang et al. 2012).

3.4.3 Inhibition of Lymphocyte Proliferation

H pylori uses the secreted proteins VacA (Gebert et al. 2003) and GGT (Shibayama et al. 2007), to inhibit lymphocyte activation and proliferation. VacA is able to inhibit proliferation of primary human B lymphocytes, as well as $CD4^+$ and $CD8^+$ T-cells (Torres et al. 2007). The VacA receptor on human immune cells is the β2 (CD18) integrin subunit (Sewald et al. 2008). In transformed Jurkat T-cells, VacA was shown to down-regulate transcription of IL-2, required for efficient lymphocyte activation and proliferation (Gebert et al. 2003). VacA does this by blocking nuclear translocation of the global regulator of immune response genes, nuclear factor of activated T-cells (NFAT). In activated primary human T-cells, VacA has also been shown to inhibit IL-2-driven cell-cycle progression independently of IL-2 secretion, by blocking the activation of regulatory proteins important for G1 cell-cycle transition (Oswald-Richter et al. 2006; Sundrud et al. 2004). Interestingly, murine T-cells, splenocytes and CD4+ T-cells do not significantly respond to VacA and this resistance is, at least in part, due to the impaired binding of VacA to murine cells (Algood et al. 2007; Sewald et al. 2008).

In common with *H. pylori* VacA, GGT inhibits proliferation of stimulated primary T-cells and peripheral blood mononuclear cells (PBMCs), but without affecting secretion of IL-2 (or IFN-γ) and without induction of apoptosis (Oertli et al. 2013; Schmees et al. 2007). The inhibition of lymphocyte proliferation involves induction of cell-cycle arrest in G1 phase through disruption of Ras-dependent signalling and requires the structural integrity of the catalytic domain of GGT and the presence of glutamine (Oertli et al. 2013; Schmees et al. 2007). It has been suggested that the inhibitory effect of GGT on T-cells is mediated indirectly by the formation of metabolites during transpeptidation (Oertli et al. 2013; Schmees et al. 2007).

3.4.4 Skewing of Adaptive Immune Responses Toward Tolerogenicity

H. pylori bacteria can manipulate adaptive immune responses to promote their persistence. One mechanism by which this may occur is via the preferential induction of regulatory T-cell (Treg) responses. This is evident in heavily colonised but asymptomatic carriers, who show Treg-predominant responses (Robinson et al. 2008). Similar findings were observed in a mouse model, in which depletion

of Tregs led to spontaneous clearance of the infection (Arnold et al. 2011). *H. pylori*-induced disease in humans was associated with low Treg responses and significantly higher levels of gastric T-helper 1 (Th1) and Th2 cells, whereas in disease-free infected subjects the balance was shifted toward elevated Tregs and a reduced T-helper response (Robinson et al. 2008). Thus, it was suggested that disease is a consequence of an inadequate regulatory response to *H. pylori* infection (Robinson et al. 2008).

DCs play a crucial role as a link between innate and adaptive immunity, being exquisitely adept at acquiring, processing and presenting antigens to T-cells. DCs present antigens in a way that promotes tolerance, at least in part, via regulation of Treg responses (Maldonado and von Andrian 2010). Priming by tolerogenic DCs converts naive T-cells into $FoxP3^+$ Tregs through antigen presentation in the absence of co-stimulatory signals or cytokines (Maldonado and von Andrian 2010). *H. pylori* is able to reprogram DCs toward a tolerogenic phenotype in vitro and in vivo. Indeed, DCs that have been exposed to *H. pylori* appear to preferentially prime Tregs over pro-inflammatory Th1 and Th17 responses and also fail to produce pro-inflammatory cytokines (Kao et al. 2010; Oertli et al. 2012; Wang et al. 2010). The importance of DCs in the development of *H. pylori*-specific immune tolerance is highlighted by the finding that systemic depletion of DCs breaks tolerance and facilitates clearance of the bacteria (Oertli et al. 2012). *H. pylori* VacA and GGT proteins play critical, non-redundant and non-synergistic roles in the tolerising effects of this pathogen on murine DCs in vitro and in vivo, by mechanisms that are independent of their suppressive activities on T-cells. Isogenic *H. pylori* mutants lacking either GGT or VacA are incapable of preventing LPS-induced DC maturation, fail to drive DC tolerisation and are attenuated for mouse colonisation (Oertli et al. 2013). Furthermore, *vacA* mutants induce stronger Th1 and Th17 responses and more severe gastric pathology (Oertli et al. 2013). VacA and GGT were reported to induce the expression of miR-155 and Foxp3 in human lymphocytes via a cAMP-dependent pathway (Fassi Fehri et al. 2010). Both VacA and GGT promote efficient induction of Tregs in vivo, while VacA is required to prevent allergen-induced asthma. The immunomodulatory effects of GGT are dependent on its enzymatic activity, whereas those of VacA are not linked to its vacuolating cytotoxicity, as strains expressing the toxigenic (s1/m1) or non-toxigenic (s2/m2) forms of VacA are equally tolerogenic in vitro (Oertli et al. 2013).

3.5 Mitigation of Inflammatory Responses

H. pylori infection leads to chronic gastritis, which generates reactive oxygen species (ROS) and nitric oxide (NO). The pathogen limits the bactericidal effects of these pro-inflammatory mediators, enabling it to chronically colonise its host. The inflammatory response induced by *H. pylori* generates large amounts of ROS, which encompass superoxide anions, hydroxyl radicals and hydrogen peroxide.

H. pylori survive these oxidative stress conditions using a variety of stress resistance proteins. These include catalase (KatA), superoxide dismutase (SodB) and three peroxidases, an alkylhydroperoxide reductase (AhpC) and two thiolperoxidases (Tpx and bacterioferritin comigratory protein, Bcp), which catalyse the breakdown of hydrogen peroxide, superoxide and organic peroxides, respectively. *H. pylori* also encodes the neutrophil activating protein (NapA), which sequesters toxic levels of iron, and NADPH quinone reductase, MdaB (Stent et al. 2012). Furthermore, *H. pylori* bacteria respond to inactivation of important oxidative stress resistance proteins by increasing the expression of their oxidative stress resistance proteins, including KatA, SodB and NapA (Olczak et al. 2005). Disruption of *katA*, *sodB*, *ahpC*, *tpx*, *bcp* or *mdaB* in *H. pylori* results in an oxidative stress-sensitive phenotype that severely affects the ability of mutants to colonise the stomach (Harris et al. 2003; Olczak et al. 2002, 2003; Seyler et al. 2001; Wang and Maier 2004; Wang et al. 2005).

Infection by *H. pylori* leads to up-regulation of inducible nitric oxide synthase (iNOS) in the gastric mucosa, leading to mucosal damage. Data obtained using cultured macrophages indicate that this induction of iNOS is dependent on the urease released from *H. pylori* (Gobert et al. 2002). *H. pylori* bacteria mitigate the bactericidal effects of NO, which is generated during the conversion of L-arginine to L-citrulline by iNOS, via the actions of an arginase, RocF (Gobert et al. 2001). The constitutively produced RocF inhibits production of NO by competing with the host for L-arginine, which is hydrolysed to L-ornithine and urea; the latter is then used as a substrate by urease. Loss of RocF activity leads to significant NO-dependent killing of *H. pylori* in vitro (Gobert et al. 2001). However, *rocF* is not essential for *H. pylori* colonisation of wild-type (McGee et al. 1999) or arginase II knockout mice (Kim et al. 2011). RocF activity is stimulated by Trx1 (HP0824), one of the two thioredoxins of *H. pylori*. Trx1 acts as a chaperone, converting denatured or suboptimally folded RocF into its catalytically active structure and reversing the damage caused by reactive oxygen and nitrogen intermediates (McGee et al. 2006).

3.6 Modulation of Apoptosis and Autophagy by *H. pylori*

Apoptosis and autophagy are intricately connected but opposing processes that can be induced in response to cellular stress and must be finely balanced to regulate cell death and survival. Perturbation of this balance can lead to pathologies such as cancer. *H. pylori* is capable of inducing and inhibiting both apoptosis and autophagy. The major known *H. pylori* virulence factors involved in these processes are VacA, CagA and GGT.

3.6.1 Apoptosis

VacA induces apoptosis of gastric epithelial cells by targeting the mitochondria, where it accumulates in the inner membrane and causes depolarisation, outer membrane permeability, cytochrome C release and mitochondrial fragmentation (Cover et al. 2003; Galmiche et al. 2000; Willhite et al. 2003; Yamasaki et al. 2006). The ability of VacA to form anion-selective membrane channels is required for cytochrome C release, mitochondrial outer membrane permeabilisation (MOMP) and cell death (Willhite and Blanke 2004). VacA induces the activation of the pro-apoptotic proteins, Bax and Bcl-2 homologous antagonist/killer (Bak), thus leading to apoptosis (Yamasaki et al. 2006). VacA-mediated MOMP and activation of Bak require the mitochondrial recruitment and hyperactivation of dynamin-related protein 1 (Drp1), a critical regulator of mitochondrial fission within cells (Jain et al. 2011). GGT induces apoptosis in gastric epithelial cells via a mitochondria-mediated pathway (Kim et al. 2007) and by inducing the loss of survivin, an inhibitor of apoptosis (Valenzuela et al. 2013). Recently, another putative *H. pylori* virulence factor, HP0986 (TNFR1 interacting endonuclease A, TieA), was reported to actively induce apoptosis in cultured human and murine macrophages via a Fas-mediated pathway (Alvi et al. 2011).

There is also evidence that *H. pylori* can prevent or block apoptosis. In a transgenic mouse model, *H. pylori* CagA was shown to interact with the apoptosis-stimulating protein of p53 (ASPP2) and thereby inhibit apoptosis by promoting proteasomal degradation of the p53 tumour suppressor (Buti et al. 2011). Host cells that had been co-cultured with *H. pylori* and then treated with the p53-activating drug doxorubicin were more resistant to apoptosis than cells not exposed to the bacterium (Buti et al. 2011). In a separate study, it was reported that CagA is also able to mediate activation of the pro-survival factor ERK and the anti-apoptotic protein, myeloid leukaemia cell differentiation protein 1 (Mcl-1) (Mimuro et al. 2007). Using the Mongolian gerbil model, the authors showed that *H. pylori* is able to activate the ERK-Mcl-1 pathway in vivo so as to suppress apoptosis in gastric pit cells, thereby leading to gland hyperplasia and persistent bacterial colonisation (Mimuro et al. 2007). In agreement with these findings, another group demonstrated *H. pylori* CagA-dependent induction of Mcl-1 expression via up-regulation of a known negative regulator of Mcl-1, the tumour suppressor microRNA (miRNA) *miR-320* (Noto et al. 2013). Consistent with the Mongolian gerbil data, *H. pylori* was shown to induce Mcl-1 expression in a CagA-dependent manner in the murine gastric mucosa, as well as in tissues from a human population at high risk for gastric cancer (Noto et al. 2013). Moreover, Mcl-1 epithelial expression levels increased at each stage of neoplastic progression in gastric tissues from human subjects infected with $cagA^+$ strains of *H. pylori* (Noto et al. 2013). Thus, down-regulation of *miR-320* and subsequent induction of Mcl-1 by $cagA^+$ *H. pylori* strains suppresses apoptosis, potentially promoting *H. pylori* persistence within the gastric mucosa but also possibly gastric carcinogenesis.

3.6.2 Autophagy

Autophagy is a tightly controlled major catabolic pathway in eukaryotes, which is required for the lysosomal/vacuolar degradation of cytoplasmic proteins and organelles. *H. pylori* induces autophagy in gastric epithelial cells (Tang et al. 2012; Terebiznik et al. 2009), as well as in professional phagocytes (Wang et al. 2010). VacA is necessary and sufficient to induce autophagy in gastric epithelial cells, with the induction of autophagy responsible for a decrease in the levels of intracellular VacA and vacuole biogenesis within intoxicated cells. Thus, autophagy may represent a mechanism by which host cells limit VacA-mediated damage (Terebiznik et al. 2009). In the AZ-521 human gastric epithelial cell line, VacA-induced autophagy was shown to be mediated by VacA binding to and internalisation via the low-density-lipoprotein receptor-related protein 1 (LRP1) (Yahiro et al. 2012). Knockdown of LRP1 abrogated VacA internalisation and significantly down-regulated autophagy in vitro (Yahiro et al. 2012). LRP1 is also required for the induction of autophagy-mediated degradation of CagA in response to m1 forms of VacA in AGS gastric epithelial cells. Signalling through p53 degradation is involved in m1VacA-induced autophagy in these cells (Tsugawa et al. 2012).

In addition to the effects of VacA in promoting autophagy, this cytotoxin can also block autophagy. Indeed, prolonged exposure of human gastric epithelial cells to VacA was found to disrupt toxin-induced autophagy, by blocking the maturation of autolysosomes (Raju et al. 2012). VacA alters the degradative properties of the endocytic pathway by subverting the sorting and activation of cathepsin enzymes (Satin et al. 1997). VacA-induced autolysosomes lack the key lysosomal hydrolase, cathepsin D, and so cannot complete the process of degrading their cargo, leading to accumulation of ROS and the signalling adaptor, p62 (Raju et al. 2012). Interestingly, impairment of autophagy and the accumulation of p62 lead to enhanced tumourigenicity (Moscat and Diaz-Meco 2009). Further work is required to determine whether VacA subversion of autophagy may promote gastric cancer development.

Finally, *H. pylori* can modulate autophagy through a mechanism involving the microRNA, *MIR30B* (Tang et al. 2012). Expression of *MIR30B* was elevated in gastric mucosal tissues from infected patients, as well as during infection of gastric epithelial cell lines, and this effect was a specific response to *H. pylori*. Moreover, elevated *MIR30B* expression in human gastric tissues was inversely correlated with the mRNA levels of the genes encoding two of the proteins that are important in regulating autophagy, autophagy-related protein 12 (ATG12) and BCL2-interacting coiled-coil protein (BECN1). Inhibition of autophagy by *MIR30B* was demonstrated to increase the number of VacA-dependent large vacuoles and enhanced the intracellular survival of the pathogen, demonstrating that autophagy is involved in regulating the levels of intracellular VacA (Tang et al. 2012). Taken together, these data show that *H. pylori* VacA is able to use different strategies to interfere with autophagy in gastric epithelial cells.

3.7 Conclusions and Outlook

H. pylori is arguably one of the most successful human pathogens, persistently colonising the gastric mucosa of more than 50 % of the world's population. This pathogen induces vigorous inflammatory host responses. However, due to the many effective strategies employed by the bacterium to subvert host immune responses, the infection cannot be easily cleared. *H. pylori* uses its numerous virulence factors to establish chronic infection, alter cellular signalling cascades, cause damage to the mucosa and modulate host immune responses. The multitude of virulence factors, together with their complex interactions and allelic variations, render it difficult to dissect the individual contributions of these factors to the chronicity of *H. pylori* infection and its long-term consequences. Moreover *H. pylori* is not only highly heterogeneous but also genetically unstable, adding to the difficulty in studying the virulence mechanisms of this human pathogen. As discussed here, *H. pylori* can alter cell proliferation, apoptosis and autophagy processes, as well as down-regulate cellular tumour suppressor genes. All together, these changes contribute to oncogenesis and the development of more severe gastric disease. Although the last decade has seen great advances in our understanding of the virulence mechanisms of *H. pylori*, much of this knowledge has been gained from experiments conducted in vitro or in animal models and awaits confirmation from clinical and epidemiological studies. Such studies must encompass populations from diverse geographic locations, as both bacterial and host polymorphisms are likely to contribute to the pathogenesis of *H. pylori* infection in humans.

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