HHS Public Access

Author manuscript

Helicobacter. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Helicobacter. 2015 September ; 20(0 1): 8–16. doi:10.1111/hel.12251.

Pathogenesis of *Helicobacter pylori* infection

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Abstract

Three decades have passed since Warren and Marshall described the successful isolation and culture of *Helicobacter pylori*, the Gram-negative bacterium that colonizes the stomach of half the human population worldwide. Although it is documented that *H. pylori* infection is implicated in a range of disorders of the upper gastrointestinal tract, as well as associated organs, many aspects relating to host colonization, successful persistence and the pathophysiological mechanisms of this bacteria still remain controversial and are constantly being explored. Unceasing efforts to decipher the pathophysiology of *H. pylori* infection have illuminated the crucially important contribution of multifarious bacterial factors for *H. pylori* pathogenesis, in particular the *cag* pathogenicity island (PAI), the effector protein CagA and the vacuolating cytotoxin VacA. In addition, recent studies have provided insight into the importance of the gastrointestinal microbiota on the cumulative pathophysiology associated with *H. pylori* infections. This review focuses on the key findings of publications related to the pathogenesis of *H. pylori* infection published during the last year, with an emphasis on factors affecting colonization efficiency, *cag* PAI, CagA, VacA and gastrointestinal microbiota.

Keywords

Colonization; CagA; *cag* pathogenicity island; VacA; microbiota

Factors affecting colonization efficiency

Shape-determining factors

The helical shape of *Helicobacter pylori* is crucial for bacterial motility and a prerequisite for successful colonization. In the last year a number of *H. pylori* genes that induce modifications in peptidoglycan cross-linking of the bacterial cell wall, or lead to trimming of peptidoglycan muropeptides have been identified. These include a number of genes that

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Competing interests: The authors have no competing interests

determine the helical shape of *H. pylori*, namely, *csd*1, *csd*2, *csd*3/*hdp*A, *ccm*A, *csd*4, *csd*5 and *csd*6. Analysis of the Csd3 structure has revealed the presence of three domains in the latent and inactive state, with the third C-terminal LytM domain containing a catalytic site with a Zn^{2+} ion, and the N-terminal domain occluding the active site of the LytM domain (1). A few months earlier, the same group investigated the structure of Csd4 and raised the possibility that Csd5 may play a regulatory role in modulating the function of Csd4 (2). Further light into the mechanism by which Csd4 can regulate bacterial shape in *H. pylori* was shed by Chan *et al*, following the study of Csd4 crystal structure and the observation that the catalytic zinc in Csd4 is coordinated by a rare His-Glu-Gln configuration that is conserved among most Csd4 homologs, which form a distinct subfamily of carboxypeptidases. Interestingly, substitution of the glutamine to histidine, the residue found in prototypical zinc carboxypeptidases, resulted in decreased enzyme activity and inhibition by phosphate (3). In addition to the influence of peptidases on bacterial cell shape through initiation of peptidoglycan relaxation, recent research has revealed that the shape of *H. pylori* may be dictated by coiled-coil-rich proteins (Ccrp). *H. pylori* contains four Ccrp (Ccrp58, Ccrp59, Ccrp1143, and Ccrp1142) and all four *ccrp* deletion mutants significantly exhibit impaired motility, despite unaltered flagella morphology. In relation to this finding Schätzle *et al*. investigated whether the deletion of Ccrp proteins could influence molecular pathogenicity in *H. pylori*, and observed a strongly decreased urease activity (4).

Motility

Motility has been shown to be essential for successful *in vivo* colonization by *H. pylori* and is provided by its sheathed flagella. In this respect Aihara *et al*. proposed that bacterial chemosensing and motility promoted very rapid *H. pylori* colonization of injury sites, thereby contributing towards sustained gastric damage, by slowing down gastric repair (5). Recently, CsrA was reported to be necessary for full motility and survival under oxidative stress, in the *H. pylori* N6 strain. Kao *et al*. reported loss of motility and lower adhesion ability for *csr* A mutants which were also shown to be non-flagellated (6). In another study, Tsang *et al*. utilizing mutational analysis demonstrated that FliO was required for wild-type motility and flagellation and that C-terminal and N-terminal regions were indispensable for regulating transcription of both *Rpo*N-dependent and *Fli*A-dependent flagellar genes (7). Moreover, their results suggested that the transmembrane region of FliO may be the most important determinant for flagellar biogenesis, being responsible for the stability of FlhA integral membrane protein of the flagellar export apparatus. In order for flagella to contribute to effective motility they require heavy glycosylation with the unusual nine-carbon sugar pseudaminic acid (Pse). Pseudaminic acid biosynthesis is a five-step pathway demanding the sequential activity of five enzymes (PseB, PseC, PseH, PseG and PseI); however only two of the required enzymes have known crystal structure, namely, PseB and PseC and therefore, it is of exceptional interest that the PseH-acetyl-CoA complex crystal structure has also been also reported (8, 9). Another study by Gauntlett *et al*. investigated whether phase-variable methyltransferases were critical for *H. pylori* colonization. Utilizing of a series of mutants in restriction-modification (RM) systems to observe variations in their colonization ability, they showed that only wild-type but not the ‘DEL’ or ‘ON’ HP1471 mutants colonized mice. Gauntlett *et al*. concluded that genome methylation and generation

of epigenetic diversity might play a dominant role in colonization and pathogenesis of *H. pylori* (10).

Chemotaxis

Bacterial chemotaxis is an essential element for successful colonization and establishment of infection, particularly for *H. pylori* populations that penetrate and grow as cell-associated microcolonies deep within the gastric glands. Chemotaxis allows a bacterium to control its swimming behavior in response to extracellular chemical signals. In a recent study Sigal *et al*. utilized advanced quantitative confocal microscopy to analyze gastric stem cell responses in infected humans and mice (11). They demonstrated that mutant bacteria with defects in chemotaxis, that were able to colonize the stomach surface, did not succeed in colonizing the antral glands in mice and also failed to activate stem cells.

The transducer-like proteins (Tlp) located in the bacterial membrane or in the cytoplasm are molecules that act as chemoreceptors of extracellular chemical signals and following interaction with their respective ligands, initiate a molecular signal transduction cascade, which causes a change in the direction of rotation of the flagellar motors. To enable screening for potential ligands and to undertake detailed structural analysis of the mechanism of ligand recognition and signal transduction, Liu *et al*. conducted cloning, overexpression, purification and crystallization of the sensory domain of TlpC of *H. pylori* (12), thereby providing a robust procedure for its purification in the soluble form.

Adherence

With regards to the stability of the binding phenotype, Nell *et al*. have suggested that following long term human infection, the ability of *H. pylori* to bind to Le^b was a relatively stable phenotype, however microevolution within the blood group antigen binding adhesion (BabA), an outer membrane protein (OMP), might occur commonly, driven by mutation and recombination, resulting in a complete loss of protein expression or in gradual changes in binding properties, thus affecting pathogenesis (13). Moreover, analyses of dynamic force spectra using an atomic force microscope revealed two distinct adhesive states, one strong and one weak, with a 5-fold variation in dissociation rates being observed respectively, suggesting that BabA might possess different domains for bacterial binding to the host ABO/Le^b binding sites in the gastric mucosa (14). Another attempt to express soluble BabA was reported by Hage *et al* in which the addition of a hexa-lysine tag at the C-terminus was shown to significantly enhance the expression and purification of BabA, without interfering with the Le^b binding (15). Such structural and biophysical characterization of the *H. pylori* BabA may provide insights into *H. pylori* adherence mechanisms and the basis for rational anti-adhesive therapeutic regimes. The gastrointestinal tract glycosylation profile is a primary factor that may determine the colonization capability of various infectious agents. Amorim *et al*. conducted glycophenotype analysis of the canine gastric mucosa, which focused on the expression of Lewis glycan antigens. This showed minor expression of type 1 Lewis antigens, with absence of Le^b and Le^a expression and only one case showing scattered expression of Le^a, offering a possible explanation as to why *H. pylori* rarely colonizes the stomach of dogs. On the contrary, they noted expression of Lewis type 2

antigens characterized by expression of Le^x and Le^y, along with a minor expression of Le^x (16).

Another novel bacterial adhesin of *H. pylori*, termed “LabA”, the encoded product of HP0025 gene in strain 26695, was identified and characterized by Rossez *et al*. (17), as it was shown to bind specifically to the GalNAcβ1-4GlcNAc motif (also known as N,N′-diacetyllactosediamine) or lacdiNAc motif located on MUC5AC mucins.

Persistence

One of the mechanisms employed by *H. pylori* for immune evasion and immune modulation is the ability to adapt to changing environmental conditions during long-term colonization. By comparative analysis of the plasticity zones in a large number of *H. pylori* genome sequences, from different genetic backgrounds, Fischer *et al*. postulated that their high prevalence and wide distribution throughout all *H. pylori* populations might provide a fitness benefit to their hosts for increased persistence (18). A further determining factor required for successful establishment of infection in such an acidic environment is active intracellular urease, a major constituent of the total bacterial protein output of *H. pylori*. In this respect, observations by Miller and Maier suggest that all four enzymes responsible for ammonium assimilation were involved in regulating either the hydrolysis of urea inside the cell, or the extent to which this ammonium was extruded versus assimilated, further supporting the association between the assimilation of ammonium and the primary acid resistance mechanism in *H. pylori* (19).

H. pylori persistence has also been demonstrated to be favored by the activity of gGT, which has been demonstrated to impair T-cell proliferation (20). More specifically, Wüstner *et al*. argued that *H. pylori* gGT can induce changes in the extracellular milieu which lead to glutamine deprivation of T-lymphocytes invading the site of infection. In relation to gGT activity in *H. pylori* infection Franzini *et al*. proposed that gGT-rich exosomes released by cancer cells, could produce within the surrounding tissues of the host effects equivalent to those for bacterial gGT, i.e., local deprivation of glutathione and glutamine, oxidative stress and suppression of T-cell immune responses (21). It is very intriguing that such gGT-dependent processes documented in bacterial virulence as well as in the biology of malignant tumors, and may indeed be a paradigm of convergent evolution, aimed at improving the survival and expansion of cellular populations in the context of a hostile resisting environment.

H. pylori Virulence Factors

cag pathogenicity island

The *cag* pathogenicity island (PAI) is widely known as the most important pathogenic factor of *H. pylori* that carries an increased risk for gastric cancer. A new report by Hanada *et al* has explained, in part, the epidemiological observation that patients infected with *cag* PAI-positive strains have twice the risk of gastric carcinogenesis as compared to those infected with *cag* PAI-negative strains. This study showed accumulation of DNA double-strand breaks (DSBs) after infection with *cag* PAI-positive strains was significantly greater than that after infection with *cag*-negative strains (*cag*A- or *cag* PAI-negative strains) (22). The

results also suggested that CagA might result in the inactivation of RAD51 and reduced activity of DSB repair via homologous recombination of host cells, after infection with *cag*-positive strains. Regarding *cag* PAI functionality in the inflammatory response to *H. pylori* infection, the *cag* PAI has been reported to be responsible for the early induction of inflammatory mediators during infection, such as chemokine CXCL1-3, CXCL5, CXCL8, CLL20, beta-defensin 2 (BD2), and tumor necrosis factor alpha (TNF-α) (23). While the *cag* PAI was highlighted as the leading factor involved in eliciting epithelial response during the early phase of infection, whereas other virulence factors appear to take over later in the development of inflammatory response. Given that the *cag* PAI encodes a type IV secretion system (T4SS) responsible for the translocation of the virulence factor CagA into the host cells and triggering a wide variety of cellular signals, the further role of T4SS in immune evasion mechanisms was recently investigated in a publication by Lina *et al*. (24). This showed that *H. pylori* uses its T4SS components CagA and peptidoglycan (PG) to upregulate B7-H1 expression in gastric epithelial cells through the p38 MAPK pathway, causing an increased T_{reg} type response, thus contributing to the establishment of a persistent infection characteristic of *H. pylori*.

Based on the observations that assembly of filamentous structures termed “pili” at the interface between *H. pylori* and gastric epithelial cells is dependent on the presence of the *cag* PAI, and the fact that CagA can be visualized at the tips of these structures, Johnson et al suggested that these structures were components of the T4SS, utilized for translocation of CagA into host cells. To investigate this issue Johnson *et al* created mutants in a set of *cag* PAI genes required for both pilus biogenesis and T4SS function. This revealed that these processes could be uncoupled based on specific mutant strains. While *cag*T, *cag*X, *cag*V, *cag*M, and *cag3* mutants were defective in both T4SS function and pilus formation, complemented mutants regained T4SS function and the capacity for pilus formation. Interestingly while *cag*Y and *cag*C mutants were defective in T4SS function they retained the capacity for pilus formation (25). This study provided evidence that mutant strains failing to produce pili were consistently defective in the *cag* T4SS-dependent phenotype, such as interleukin (IL)-8 induction in gastric epithelial cells.

H. pylori-mediated diseases are mainly due to the effect of bacterial virulence factors, thus, understanding the biological features and mechanisms of these factors may provide a more comprehensive insight in the pathogenesis of *H. pylori* infection. In light of new findings, the function of the T4SS for delivery of CagA into the host cells has been reported for the first time to be turned-off by the Y58/E59 mutation in *cag*L (26). Located at the end of a loop between α1 and α2 helices, the highly flexible region of the *cag*L, Y58/E59 motif, has been shown to affect the properties of this region and to alter the position of α1, thus controlling integrin binding through burial or exposure of Arg76 of the *cag*L RGD motif, a feature, essential for integrin binding and translocation of CagA (26, 27). Moreover, the observation of pH-mediated conformational changes being critical for the structure of the CagL protein, revealed a novel molecular mechanism for regulating integrin engagement by RGD motif (27). The presence of proteins CagX and CagT also contribute in the constitution of a functional T4SS such as in pilus formation, translocation of CagA or induction of IL-8 and in contrast with previous observations, the direct interaction between these proteins has

been addressed in a recent publication (28). The authors determined less expression of CagT in the absence of CagX and that the C-terminal 200 residues of CagX may be important for CagT interaction.

CagA

During *H. pylori* infection, CagA is translocated into the host cells via the T4SS and has been shown to activate various oncogenic pathways in the host cell. New data by Wei *et al*. suggested that tumorigenicity associated with *H. pylori* infection was linked to inhibition of p53 protein by CagA (29). The authors proposed a model in which CagA-induced degradation of p53 protein was determined by a relative level of p14ARF tumor suppressor and that the p14ARF protein counteracts *H. pylori*-induced signaling. Interestingly, although CagA is a crucial component of the T4SS, it has recently been suggested that CagA could activate separate pathways with the T4SS. By comparing signaling changes over time and in the absence of CagA or the T4SS, this study provided evidence of distinct pathways of phosphotyrosine to be induced by CagA and T4SS. While CagA mainly was found to induce phosphorylation of extracellular signal-regulated kinase 1 (ERK1) and integrin-linked factors such as breast cancer 1 (BCAR1), the rest of the T4SS components induced phosphorylation predominantly of c-Jun N-Terminal Protein Kinase (JNK) 2/3 and p38 (30).

Biological activity of CagA is determined by variation in the number and sequences of Glu-Pro-Ile-Tyr-Ala (EPIYA) tyrosine phosphorylation motifs in C-terminal regions. Four distinct CagA EPIYA motifs, East Asian type CagA include EPIYA-A, -B, and usually only one EPIYA-D, while Western type CagA include EPIYA-A, -B, and -one or multiple EPIYA-C motifs have been reported. Among Western type strains, the number of EPIYA-C motifs has been considered to be the most important risk factor for developing cancer. Based on analysis of 42 identified key genes of signal transduction involved in gastric cancer at the transcription level, new insights into the assessment of the effects of the number of EPIYA-C motifs on host cellular fate have been revealed. Varizi *et al* have demonstrated that ABCCC oncoprotein variant can change the fate of the cell completely different from ABC type. These results of the study proposed that ABCCC type can induce the intestinal metaplasia, IL-8, perturbation of Crk adaptor proteins, anti-apoptotic effect and carcinogenic effect more significantly than ABC type, whereby variations in number of the EPIYA-C motifs were believed as marker in determining the host cellular fate (31). With regards to the regulation of CagA activity related to carcinogenesis, a further report has highlighted the contribution of the A/T polymorphisms of the EPIYA-B motif (32). During co-culture with AGS gastric epithelial cancer cells, an *H. pylori* strain with an EPIYT-B showed significantly attenuated induction of IL-8 and the hummingbird phenotype as compared to the isogenic strain with the EPIYA-B. This suggests that among Western type CagA strains, the EPIYA-B motif is significantly more associated with gastric cancer than the EPIYT-B motif. Compared to Western type CagA, East Asian type CagA have been authenticated as being more interactive with host cells and more strongly associated with severe clinical outcomes. Investigation of clinical samples obtained from patients in Bhutan, a recent publication has indicated that IL-8 mRNA levels are significantly higher in biopsies of patients infected with East Asian type CagA strains than those with Western type CagA strains, even among participants with mild infiltration of polymorphonuclear leucocytes

(PMNs). In addition, despite higher IL-8 mRNA levels, relatively low PMN responses were observed in this Bhutanese population who area predominantly infected with East Asian type CagA strains (33). These findings corroborated again the notion that East Asian type CagA strains are associated with increased expression of IL-8, as compared with Western type CagA strains, but in some cases are less effective in eliciting a PMN response.

VacA

As one of the major virulence factors in the pathogenesis of *H. pylori* infection, VacA is well known as a secreted, pore-forming toxin, present in almost all strains; however the mechanisms by which VacA are secreted remain undefined. It has previously been believed that VacA secretion is regulated by a T5SS in which C3G inhibits synthesis of SecA, resulting in the inhibition of VacA secretion (34). However a recent study has highlighted that an acidic pH in the extra-bacterial environment can stimulate an intra-bacterial nanotransportation system (*ib*NoTS) for priming VacA secretion, and that this novel type of *ib*NoTS for VacA differs from that for CagA or Urease (35). Following internalizing into cells, VacA can cause multiple cellular alterations, including cell death. Although the mechanisms by which VacA causes cell death are still under debate, a report has now provided strong evidence that VacA-mediated death of gastric epithelial cells may be through a Cx43-dependent pathway (36). Cx43 is the most common connexin isoform and contributes to VacA-induced cell death, without direct interaction with VacA. In a more recent study, VacA-induced apoptotic cell death was found to be regulated by endoplasmic reticulum (ER) stress signaling (37). The induction of ER stress signaling, the result of dendritic cell (DC) stimulation with VacA, led to the release of cytochrome *c* and DNA fragmentation, leading to increased apoptosis of DCs. In contrast, suppression of ER stress resulted in a significant inhibition of the VacA-induced apoptosis in DCs. A further distinct VacA effect is its contribution to persistent infection by *H. pylori* through inhibiting the proliferation and immune response of T cells (38). During *H. pylori* infection, T cells are practically hypo-responsive, due to the activity of transforming growth factor β (TGF-β) exerting a suppressive effect on T cells. Moreover, the expression levels of mucosal TGF-β1 have been demonstrated to be dependent on *vacA* genotypes, with a positive correlation being reported between secreted *vacA* s1 (or s1m1) types and increased mucosal TGF-β1 mRNA levels and increased mucosal TGF-β1 mRNA levels thereby, contributing to persistent infection.

Because nearly all strains isolated from humans are positive for VacA, genotypes of *vac*A are documented as the critical determinant of pathogenesis, rather than its presence or absence. In order to characterize the role of different *vac*A genotypes in *H. pylori* pathogenesis, Winter *et al*. observed the different levels of *vac*A genotypes associated-gastric damage in infected-humans and -mice (39). The association between *vac*A genotypes and inflammation was only observed in infected mice and interestingly, compared with the less-active *vac*A s2/i2 strains, strains producing s1/i1 and s1/i2 *vac*A showed reduced colonization rates and densities. Further non-*vac*A i1 strains were shown to cause mild metaplasia. In humans the *vac*A i1 was strongly associated with moderate to severe metaplasia, with an almost complete absence of intestinal metaplasia being found in subjects infected with *vac*A i2 strains, even among the *vac*A s1 and *cag*A positive background (36).

In addition to associating with precancerous intestinal metaplasia, *vac*A s1 and i1 genotypes have been reported to be a better marker of gastric cancer- and duodenal ulcer- associated *H. pylori* strains than *cag*A status, or the size of the *cag*A 3′ variable repeat region (40). Furthermore, a later study in Pakistan has reported that most *H. pylori* isolates found to be resistant to all antibiotics were positive for the *vac*A s1a/m1a genotype (41). Taken together, these results accentuated the decisive role of *vac*A polymorphic form in *H. pylori* pathogenesis.

Concerning *H. pylori*-related extradigestive diseases, a study has demonstrated for the first time that VacA was present in human lung tissues and was more prevalent in lungs of patients with collagen vascular disease-associated interstitial pneumonia than in those of patients with idiopathic pulmonary fibrosis, nonspecific interstitial pneumonia and cryptogenic organizing pneumonia (42). The findings of the study suggested that induction of IL-8 and IL-6 in airway epithelial cells had specific reaction against VacA stimulation. Consistently with this finding, a study on the specific relationship between smoking and bacterial load has noted the evidence of the association of *vac*A i1 type with active-smoking (43).

Other bacterial virulence factors

The contribution of bacterial virulence factors to *H. pylori*–host interactions has been recently reviewed (44) and the necessity for the use of more representative populations in epidemiologic studies associating particular virulence factors with a clinical outcome has been emphasized. Moreover, the need to examine putative virulence factors as part of a virulence complex rather than individually is highlighted, as it is expected to provide a more comprehensive and accurate understanding of the pathogenic mechanisms.

The outer inflammatory protein (OipA), an OMP, is a major virulence factor in *H. pylori*, that in synergy with other virulence factors, plays a role in the induction of key pro-inflammatory molecules such as IL-8, the activation of key components which regulate stress fiber activation and cellular morphology as well as β-catenin signaling, affecting intercellular junction formation and proliferation. In a recent study Teymournejad *et al*. examined the effect of purified OipA on the maturation and cytokine production by dendritic cells (DC) (21). Key DC maturation markers CD40, CD86, and MHCII were found to be moderately down-regulated in OipA-treated DCs at the high end range of OipA concentrations used (10 and 20 μg/mL). However, production of IL-10 was decreased over the whole range of OipA concentrations used. In contrast no change in IL-12 was detected, suggesting that OipA may act as a DC-maturation suppressor thereby assisting the establishment of chronic infection (21).

Following on from recently published reports on the complex regulation of another OMP, sialic acid binding adhesion (SabA) expression in *H. pylori*, an additional investigation into repetitive sequence variations in the promoter region of SabA has been published and has not only provided a more in depth understanding of the control of SabA expression, but also its critical role in disease pathogenesis (45). This study by Harvey et al identified when the poly(T) tract length in the *sab*A promoter region was increased or decreased by five nucleotides and there is a maximal differential effect on *sab*A expression. The interaction

between the *sab*A promoter region and ArsR, a response regulator affecting *sab*A expression was characterized, and the ArsR binding site of SabA was determined to be downstream of the poly(T) tract and the transcriptional start site. The study led to the conclusion that ArsR functioned as a *sab*A repressor by preventing passage of the RNA polymerase independent of the poly(T) tract. Concerning the function of surface-exposed OMPs in mediating bacterial-host interaction and the cell surface topology, Voss *et al* reported that most of the protease-susceptible OMPs contain a large protease-susceptible extracellular domain, that is exported beyond the outer membrane, as well as a protease- resistant domain, located at the C terminus with a predicted-barrel structure (46). This publication also suggested that, similar to the secretion of the VacA passenger domain, the N-terminal domains of protease-susceptible OMPs can be exported through an autotransporter pathway.

The ability of *H. pylori* to modulate key functions of macrophages, a key cellular component of the host immune response system in an attempt to establish persistence of the infection, has been highlighted by two further studies. Tenguria *et al*. suggested that the product of the bacterial gene *JHP0940*, namely CtkA, a cell-translocating Ser/Thr kinase could also act as an auto-phosphorylation tyrosine kinase, inducing an apoptotic effect on a mouse macrophage cell line, through Fas expression, mimicking the activity of the mammalian kinase, c-Abl (19). In another report, Du *et al*. highlighted that cholesterol-α-glucosyltransferase, the product of the *cap*J gene in *H. pylori*, has been shown to contribute significantly to the delayed bacterial entry into the macrophage and pronounced arrested phagosome maturation (47). Furthermore, Du *et al* suggested that cholesterol glucosylation by *H. pylori* modulates the lipid-raft trafficking endocytic pathway, that requires PI3K activation, thereby favoring prolonged bacterial survival inside macrophages. Finally, Nisimova *et al* investigated the role of *H. pylori* infection in the phosphorylation of pro-apoptotic factor Raf kinase inhibitor protein (RKIP)(48). They concluded that *H. pylori in vitro* and *in vivo* infection can induce phosphorylation and subsequent activation of RKIP which in turn can downregulate pro-tumorigenic factors such as EGFR, Raf-1, and MAPKs and therefore effectively attenuating pro-survival signals in gastric tumorigenesis.

Microbiota and *H. pylori* infection

The outcome of *H. pylori* infection is thought to be dictated by host genetic factors, bacterial virulence determinants and environmental components such as salt consumption, smoking and living conditions (49). However, the more recent acknowledgment of the importance of the gastrointestinal microbiota has added another potential determinant, the composition or structure of bacterial communities in the stomach, either at the time of exposure or over the course of infection (50). A number of studies have examined putative changes in the microbiota of the gastrointestinal system as a result of *H. pylori* infection and/or eradication, utilizing animal infection models. For example Khosravi *et al*. have investigated the interplay between *H. pylori* and normal gut microbiota during early stages of life using germ free (GF) and specific pathogen free (SPF) mouse models (51). This study showed that *H. pylori* could interact with normal gut microbiota, which in turn could alter metabolic and inflammatory pathways in *Helicobacter*-infected animals as compared to uninfected animals. Moreover, Khosravi *et al* postulated that as this ongoing crosstalk between *H. pylori* and the normal gut microbiota is associated with gut inflammatory responses, the

onset of *H. pylori* mediated diseases in humans might be much more complex. In a further study Whary *et al*. employed the gastric cancer-promoting INS-GAS mouse model to study the impact of a helminth infection, caused by *Heligmosomoides polygyrus*, on *H. pylori*-associated gastric lesions and microflora (52). Most notably, their conclusions supported the importance of enteric colonization in promoting *H. pylori*-associated gastric cancer in INS-GAS mice. Although similar gastric inflammation and high levels of proinflammatory mRNA was documented, helminth co-infection significantly reduced *H. pylori*-associated gastric atrophy, dysplasia and prevented *H. pylori*-induced changes in the gastric flora. In addition Heimesaat *et al*. have investigated potential changes of the microbiota composition distal to the inflammatory process, particularly in long-term *H. pylori* infected Mongolian gerbils (53). This showed that in the proximal part of the small intestine (duodenum, jejunum) no difference was observed in the luminal microbiota composition of *H. pylori* infected versus non-infected gerbils. However, in the ileal lumen of *H. pylori* infected animals, 1.5 orders of magnitude lower numbers of *Lactobacillus* spp. were detected as compared to the uninfected animals or those infected with a T4SS-defective *H. pylori* strain. In caecum and colon *H. pylori*-infected gerbils harbored significantly higher *E. coli* and *Enterococcus* spp. loads compared to naive animals, yet in a T4SS-depended manner. Taken together these results suggest that *H. pylori* induced gastric immunopathogenesis in the form of hypochlorhydria and/or hypergastrinemia, might trigger large intestinal microbiota changes, especially in the distal uninflamed gastrointestinal tract. In another study, Shiu *et al*. investigated the ability of lymphoid tissue inducing (LTi) cells to regulate pathogen, as well as commensal populations, by promoting antimicrobial peptide production in the gastric epithelium, in wild-type and IRAK-M knockout mice, infected by *H. pylori* or *H. felis* (54). This showed that IRAK-M could limit *H. pylori*-associated lymphoid follicle formation and that *Helicobacter*-associated lymphoid follicle formation was regulated independently of inflammation. Moreover, Helicobacter infection induced LTi cell-dependent antibacterial peptide production did not elicit significant changes in the gastric microbiome or promote eradication of *Candida albicans* from the gastric mucosa. Finally, Tian *et al*. investigated microbiota changes in the lower esophagus, by DGGE profile analysis of the 16S rDNA V6 region, following successful eradication of *H. pylori* infected mice by administration of antibiotics (55). This showed an increased number of bacterial species including *Acinetobacter*, *Klebsiella* and *Enterobacter species* to be present in the lower esophagus of infected mice as compared to the control and eradicated groups. These findings warrant further investigation into the role the lower esophageal microbiota on esophageal disease.

Two studies have focused on the analysis of gastric microbiota in patients with *H. pylori*-positive or negative gastric disease (56, 57) to determine whether *H. pylori* colonization affects the diversity of the gastric microbiota. These studies showed conflicting results possibly due to differences in the methodology used (MALDI-TOF analysis of cultured isolates vs. 16S rRNA gene amplification and pyrosequencing). Finally, a number of reports have focused on the isolation of lactobacilli with probiotic properties, from healthy individuals (58, 59). Moreover, Delgado *et al* proposed a number of properties such as acid resistance, bile tolerance, adhesion to epithelial gastric cells, production of antimicrobial compounds, inhibition of *H. pylori* in *in vitro* and *in vivo* animal models, antioxidative

activity, antibiotic resistance, carbohydrate fermentation, glycosidic activities, and the ability to grow in milk, as a minimum list of functional and technological properties for the determination of suitable candidate probiotics.

Conclusion

In summary, the findings in the field of *H. pylori* pathogenesis raised over the last year provide a better understanding of persistence, as well as, of the pathophysiological mechanisms of *H. pylori*. However, further studies in this field are necessary to allow a deeper understanding of *H. pylori*-mediated diseases and the development of novel interference.

Acknowledgments

Disclosures: We sincerely apologize to all authors in the field who have published on *H. pylori* pathogenesis during the past year, whom we could not cite in this review, due to page limitations. This report was based on work supported in part by grants from the National Institutes of Health (DK62813) (YY) and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (25293104, 26640114 and 15H02657) (YY) and the InfeNeutra project, KRIPIS National Strategic Reference Framework (ESPA 2007-2013, MIS 450598) of the Ministry of Culture and Education of Greece (DS).

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