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REVIEW: PATHOGENESIS OF *HELICOBACTER PYLORI* INFECTION

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Abstract – In this article we summarise recent data published on the mechanisms involved in the complex interaction of *Helicobacter pylori* with the gastric mucosa as well as cellular compartments beyond the stomach. This includes the direct effect of *H. pylori* membrane proteins and their interplay with epithelial surface proteins, mediating cellular adhesions and facilitating colonisation. Another highly relevant factor remains the modulation of different immune cell types, not only having an effect on both local (gastric) and systemic inflammation, but also having been suggested to be relevant in the pathogenesis of non-gastric conditions such as arteriosclerosis and Alzheimer's disease. Direct cell-cell interaction of the *H. pylori* stimulated gastric microenvironment, including activation of nuclear factor kappa B dependent signalling in epithelial and inflammatory cells, regulates cell proliferation and migration in the process of gastric carcinogenesis. Both host and bacterial genetic factors that influence these processes remain in the focus, but there is also increasing interest in how these factors alter the composition of not only the gastric, but also the intestinal microbiota and what effect this has on the individual. Finally, resistance of *H. pylori* to antibiotics has become an increasingly relevant threat since treatment failure can lead to a significant long-term risk for the patient. Hence, mechanisms involved in resistance development are a major focus of current *H. pylori* studies.

Keywords: *Helicobacter pylori*, Outer membrane protein, Bacterial colonisation, Toll-like receptors, Gastric carcinogenesis, Immune-modulation, Antibiotic resistance, Gastric microbiota.

INTRODUCTION

Forty years after its discovery, *Helicobacter pylori* remains one of the bacteria with the most relevant impact on human health. The complex interaction of the bacteria and its manifold bacterial virulence factors with diverse cell types both in the stomach and beyond is still the focus of intensive investigation. In this article, we summarise some of the data on the mechanisms involved that have been published in the last year. PubMed was searched for all articles indexed for '*Helicobacter*' during the period between April 2021 and March 2022. Article titles were then screened and all studies that presented new insight on mechanisms involved in pathogenesis of *H. pylori* associated conditions were selected. We focused on original studies that were published in English. The remaining articles were then grouped under topics as provided by the headings within this article. This means that single individual papers were not selected for this review.

HELICOBACTER PYLORI AND GASTRITIS

Interaction of *H. pylori* with the gastric epithelium

H. pylori is well known for its ability to induce chronic inflammation in the gastric epithelium. However, it is still not fully understood how *H. pylori* can survive in such a hostile microenvironment. The initial step of the infection is marked by the adhesion of *H. pylori* to gastric epithelial cells. Numerous molecules are involved in this process. Recent studies have emphasised the role of outer membrane proteins (OMPs) such as Helicobacter outer membrane A and B (HomA and HomB). Tamrakar et al[1] offered new insight into the structural aspects of these proteins by X-ray scattering techniques and revealed that they consist of discontinuous N and C terminal β-strands forming a small β-barrel, similar to OMPs of other Gram-negative bacteria1. Furthermore, these proteins seem to form a surface-exposed globular domain.

Another vital OMP for *H. pylori* adhesion is the OMP Q (HopQ). Its interaction with the human carcinoembryonic antigen-related cell adhesion molecule (CEACAM) on gastric cells has been studied extensively. However, it has been established to be a relevant virulence factor in its own right. Taxauer et al[2] demonstrated that HopQ-CEACAM interaction is vital for inflammatory processes since its expression levels correlate with non-canonical NF-kB activation in cell culture, as well as in human gastric tissue samples[2].

Colonisation of the gastric epithelium is further mediated by gastric surface adhesion molecules such as Lewis antigens that interact with the surface proteins of *H. pylori*, *e.g.* blood group antigen-binding adhesion (BabA) and sialic acid-binding adhesin (SabA). Recent studies suggested an influence of bacterial density on a differential immune response in children and adults. To further investigate the role of adhesion molecules in this matter, Yang et al[3] used human stomach foetal epithelium (HSFE) and SV40-immortalised human normal gastric epithelial (GES-1) cell lines as a model for the gastric epithelium in children and adults, respectively. As expected, the intensity of *H. pylori* colonisation correlated with the expression of Lewis antigens, which was higher in GES-1 cells. In turn, the expression levels of IL-6 and IL-8 were higher in HSFE cells. Yang et al[3] postulated that mitogen-activated protein kinase (MAPK) activation is responsible for this effect since activation of p38 mitogen-activated protein kinases (p38) and extracellular-signal regulated kinases (ERK), that are both subfamilies of the MAPK pathway, was more present in HSFE cells. A higher p38 activity in the gastric epithelium could suppress c-Jun N-terminal kinase (JNK) expression and possibly lead to the lower Lewis antigen expression and, hence, lower bacterial density in children.

Interaction with other cell types and related metabolic factors

An *H. pylori* infection triggers a complex inflammatory response involving a vast amount of different mediators that manipulate the host's mucosal defence. This leads to a radical change in the mucosal microenvironment enabling chronic infection. Toll-like receptors (TLRs) are one of the key stimulators of the innate immune system and play a major role in early inflammation, also directly influencing susceptibility to *H. pylori* infection.

Schmidinger et al[5] elucidated how binding of human annexins (ANX) could suppress LPS-mediated Toll-like receptor 4- (TLR4) signalling. They identified the lipid A portion of *H. pylori* LPS as a binding target for human ANXA5 leading to a reduced surface charge. Since structural and experimental studies showed that especially the 4´-phosphate group of lipid A is important for TLR4 recognition, this could be an effective way to evade the innate immune system.

Hernandez et al[6] demonstrated an activation of cytotoxic natural killer (NK) cell function via TLR4/MyD88 (myeloid differentiation primary response 88) dependent pathways. This involved natural killer group 2 member D (NKG2D) ligands which were upregulated both under stress conditions in *in vitro* settings as well as in the process of malignant transformation of the gastric mucosa.

Wen et al[7] studied the role of TLR2 in *H. pylori* mediated production of reactive oxygen species (ROS) and secretion of IL-8 in neutrophils. For this purpose, they created all-trans-retinoic acid (ATRA)-induced, differentiated HL-60 cells as a model for neutrophils and exposed

them to *H. pylori* neutrophil-activating protein (HP-NAP). In fact, blocking TLR2 with neutralising antibodies did not affect the production of ROS, but attenuated the secretion of IL-8 in neutrophils. This suggests that HP-NAP induced production of IL-8 is indeed mediated by TLR2.

As a response to infection, gastric epithelial cells secrete numerous different cytokines in order to induce inflammation and to attract immune cells. The *cag* pathogenicity island encoding for a type four secretion system (T4SS), as well as VacA and urease, is one of *H. pylori*'s main bacterial virulence factors. However, there are multiple further factors that orchestrate the host's immune response[8].

Tumour necrosis factor (TNF)-alpha inducing protein alpha (Tipα) secreted by *H. pylori* induces the production of TNFα and other pro-inflammatory cytokines via NFκB) dependent pathways. By infecting mice with *tip*α-knock-out strains compared to wild-type Sydney strain (SS1), Morningstar-Wright et al confirmed a reduced production of Interferon (IFN)γ and TNFα suggesting that Tipα is of relevance in Th1-associated inflammation9. Furthermore, Tipα might play a role in the development of gastric hyperplasia since mice infected with knock-out strains showed significantly lower hyperplasia scores after four months compared to those infected with a wild-type strains.

Recent studies[10-12] started to elucidate the role of heptose metabolites in *H. pylori* virulence, focusing on adenosine diphosphate heptose (ADP-heptose), a metabolite in lipopolysaccharide (LPS) synthesis. Faass et al[10] studied the role of ADP-heptose in the activation of mononuclear phagocytes (*i.e.* monocytes, macrophages) by exposing THP-1 cell line cells to pure ADP-heptose in the absence of other microbe associated molecular patterns (MAMS). This led to strong activation of monocytes associated with a specific transcriptome profile and triggered proinflammatory M1-differentiation of macrophages. This effect was significantly reduced in TNF receptor associated factor (TRAF)-interacting protein with a *forkhead-associated domain* (*TIFA*) knock-out cells, consistent with previous findings that suggested a direct interaction of ADP-heptose with the alpha-protein kinase 1 (ALPK1)-TIFA pathway, eventually activating NF-κB.

Maubach et al[11] focused on the same pathway in gastric epithelial cells. They described the formation of a 'TIFAsome' with TRAF6 and TRAF2 upon ADP-heptose activation and analysed the impact of those two proteins on NF-κB activation using knock-out models. Interestingly, TRAF2 and TIFA induced activation of classical NF-κB signalling through TGFβ-activated kinase 1, whereas TRAF6 and TIFA contributed to the activation of the alternative NF-κB pathway by its involvement in the degradation of cellular inhibitor of apoptosis protein 1 (cIAP1). This suggests the formation of two different TIFAsomes and the activation of both NF-κB pathway variants leading to gastric pathologies induced by *H. pylori* infection.

Coletta et al[12] described another effect of ADP-heptose on chronic inflammation. They demonstrated that macrophages exposed to ADP heptose in cell culture expressed reduced levels of class II major histocompatibility complex transactivator (CIITA), the key regulator of human leukocyte antigen (HLA)-II-genes. Consequently, macrophages showed reduced levels of HLA-II surface expression. The expression of HLA-II was rescued by NF-κB inhibition, which also led to reduced IL-8 levels. This illustrates once more the involvement of ADP-heptose on NF-κB activation and its role in chronic inflammation.

Other targets under scrutiny are those influencing cytoskeleton formation and hence plasticity and mobility of gastric cells. Investigations of the effect of a CRISPR/Cas9 mediated knockout of cortactin in gastric AGS cells revealed a clear interaction of the molecule with intracellular phosphorylases and hence the effect of bacterial virulence factors of *H. pylori*[13,14]. Cortactin knockout leads to diminished phosphorylation of intracellular CagA by reducing the activity of focal adhesion kinase (FAK), and tyrosine Src and Abl kinases. This has further effect on cytoskeletal re-arrangement, endosomal trafficking and cell migration.

Further factors relevant to bacteria-host interaction

While some single nucleotide polymorphisms (SNPs) in the host's and the bacteria's genome, such as in TLR or LPS, have been described to influence susceptibility or virulence respectively, a Chinese study performed a genome-wide association study on a population of 480 people.

They identified three genetic regions associated with *H. pylori* infection status and 14 associated with the bacterial load. Moreover, they identified several pathways involved in immune response[15]. Of these, variations in the glycosaminoglycan biosynthesis pathway, which plays a role in bacterial adherence, had the strongest effect on susceptibility to *H. pylori*. A study by Barakat et al[16] on a small population of children could identify polymorphism of the *IL-1B* gene at position -511 as a risk factor for *H. pylori* infection and severe corpus gastritis, similar to previously reported data in adult populations.

Several virulence factors of *H. pylori* have been characterised in the past and their impact on gastritis has been analysed extensively. Those studies concentrated mainly on *cag*A, *cag*E, *ice*A1, *ice*A2, outer membrane inflammatory protein A (*oip*A) and *bab*A2. A strong association with peptic ulcer was reported for *oip*A, *bab*A2 and *vac*A, while *ice*A1^{-}, *bab*A2+, and *oip*A+ genes were associated with high scores for colonisation density[17]. In a Turkish population of dyspeptic patients, CagA and BabA2 were the most abundant virulence factors[18].

Recent studies19,20 also identified new virulence factors. Hsu et al[20] described the important role of cholesterol-α-glucosyltransferase in bacterial-epithelial adhesion by enabling the formation of lipid rafts19. In contrast, a Brazilian study challenged the status of *dup*A as a virulence factor since there has been no correlation with gastritis aligning with previous studies20.

HELICOBACTER PYLORI AND GASTRIC CARCINOGENESIS

Several studies[21-24] addressed the interaction of *H. pylori* with the epithelial cell compartment and the mucosal microenvironment with emphasis on its effect on gastric carcinogenesis.

Cao et al[21] investigated targets of Wnt/β-catenin signalling both *in silico* and in mouse models. By bioinformatics analysis of both publicly available and locally generated datasets, they identified RAS protein activator like 2 (RASAL2) as a target that is capable of β-catenin transcription. RASAL2 expression is induced by *H. pylori via* NF-κB dependent signalling. RASAL2 inhibits protein phosphatase 2 by direct binding, hence activating the Akt/β-catenin axis. Silencing of RASAL2 inhibited formation of cell spheroids *in vitro* as well as the formation of patient derived organoids. There was also less tumour growth in a respective mouse model. Analysis of data on the expression of RASAL2 in human gastric cancer tissue shows a correlation with poor prognosis and chemoresistance[21].

A further factor influencing gastric tumourigenesis via the Akt/NF-κB axis is differentiated embryo-chondrocyte expressed gene 1 (DEC1). DEC1 is upregulated in gastric cancer tissue and also correlates with cancer progression and poor prognosis[22]. The effect of DEC1 dependent cell proliferation was confirmed in a mouse model.

Another relevant factor is cytokine induced cell stimulation. Aziz et al[23] demonstrated that IL-10 knock-out in mice with either an *H. pylori* infection or alcohol induced gastric cancer, or both had accelerated tumourigenesis. The animals presented CD8+ T cells with damaged mitochondria leading to an accumulation of IL-1 β. When IL-10 was reintroduced, then cellular glucose uptake and glycolysis was inhibited, whilst in parallel promoting oxidative phosphorylation and lactate inhibition.

CagA positive *H. pylori* infection upregulates expression of C-X-C motif chemokine ligand 8 (CXCL8) which mediates cell proliferation and migration both *in vitro* as well as enhanced tumour growth *in vivo*[24]. This process is enhanced by knock-down of Kruppel-like factor 4 (KLF4). Interestingly, CXCL8 inhibits KLF4 expression by direct promotor binding.

Aydin et al[25] demonstrated that the upregulation of programmed cell death protein 1 and its ligand (PD-1 and PD-L1) in gastric ulcers and gastric cancer is more pronounced in correlation with *H. pylori* density. They also present a complex pattern of differential association of both PD1 and PD-L1 to various bacterial virulence factors of *H. pylori*, *e.g.*, a negative association of PD-L1 with the presence of CagA positive strains in gastritis, but a positive association of PD1 with strains carrying the vacA s2/m2 genotype.

Chen et al[26] focused on activing A receptor type 1 (ACVR1), a protein that is upregulated in both intestinal metaplasia (IM) and gastric cancer. While *H. pylori* can stimulate ACVR1 expression, this is independent from CagA. In vitro data from AGS cells with CRISPR/CAS knock-out of ACVR1 and data from *H. pylori* infected C57/BL6 mice shows that *ACVR1* knock-

out has a negative effect on *H. pylori* induced activation of p-Smad (Smad family member) 1 and 5, Cdx2 Caudal type homeobox protein 2) and the bone morphogenetic protein (BMP) signalling pathway.

Other studies[27,28] showed an influence of *H. pylori* infection on the expression of epigenetic regulators, including DNA-methyltransferases (DNMT1, 3A and 3B) and a differential pattern of genetic methylation in *H. pylori* positive subjects.

Another factor that has received a lot of interest is the interaction of *H. pylori* induced inflammation and bile acids in the stomach. Noto et al[29] studied transgenic insulin-gastrin (INS-GAS) mice under iron deficient conditions. These animals showed a significantly altered bile acid profile with upregulation of deoxycholic acid (DCA). Treatment of mice with DCA had a procarcinogenic effect also facilitating CagA translocation in epithelial cells. The severity of mucosal lesions correlated with expression of the G-protein coupled bile acid receptor 5 (TRG5). Administration of bile-sequestrants hindered this process. Interestingly, the analysis of data from 416,885 individuals showed a dose-dependent reduced risk for gastric cancer in patients on treatment with bile-sequestrants.

HELICOBACTER PYLORI AND IMPACT ON OTHER SYSTEMS

Influence on other diseases

While former studies often focus on the influence of *H. pylori* on the gastric mucosa, recent studies[30-35] indicate an even broader effect on the host. Increasing evidence suggests a role of *H. pylori* in arteriosclerosis. Extracellular vesicles containing CagA were measured in blood samples of infected mice and patients to assess the impact of *H. pylori* status. Accordingly, atherogenesis was associated with CagA positive *H. pylori* strains, but not with CagA negative strains as shown by Li et al[30].

Moreover, CagA seems to promote the expression of adhesion molecules and inflammatory cytokines of aortic endothelial cells in a murine model. Krupa et al[31] could show that exposing macrophages to soluble *H. pylori* components *in vitro* induced their differentiation into foam cells, which are involved in the development of atherosclerotic plaques.

Although epidemiological studies[32] suggested an association between *H. pylori* infection and Alzheimer's disease, little is known about the potentially underlying molecular mechanisms. Iwasaki et al[33] demonstrated *in vitro* that HpHpn, a histidine rich protein secreted by *H. pylori* with the ability to form amyloid-like oligomers, is taken up by gastric epithelial-like carcinoma cells. The ability of HpHpn to pass the blood-brain barrier *in vitro* suggests its potential as a systemic virulence factor. Ju et al[34] suggested a connection between metabolic dysfunction and Alzheimer's disease, since they could induce metabolic dysfunction in mice by faecal transfer from Alzheimer's patients. *H. pylori* might play an important role in this mechanism by causing increased gut permeability through activation of TLR4/Myd88 dependent pro-inflammatory signalling[34]. These studies give an interesting insight into the potential of *H. pylori* as a systemic pathogen. However, more research is necessary to verify these findings.

Effect on the Microbiota

Wang et al[35] sequenced gastric mucosal swab samples in order to analyse the impact of *H. pylori* infection on the gastric microbiota. In this Chinese population of 96 people, the authors detected a depletion of the α-diversity, meaning a decrease in species diversity, and a dominant position of *H. pylo*ri in those positive for the infection. Mao et al[36] reported a similar decrease of diversity upon *H. pylori* infection in a study of 89 people and demonstrated a change in the gastric microbiota after eradication towards a state similar to that of *H. pylori* negative patients. In both studies *H. pylori* infection was associated with the abundance of other species. While Mao et al[36] could detect an increase in *Curvibacter* and *Acinetobacter* genera upon *H. pylori* infection, Wang et al[35] identified other infection-associated species, like from the *Stenotrophomonas* and *Chryseobacterium* genera. Recent studies[37,38] have elucidated a possible impact of *H. pylori* on the gut microbiota. A small study in the USA showed

a reduction of the alpha diversity in faecal samples of *H. pylori* positive patients over 40 years of age compared to controls of similar age[37]. This effect was confirmed in a Japanese study on adolescents[38]. Despite these more associative results, the molecular mechanisms underlying these effects are still not clear.

HELICOBACTER PYLORI ANTIBIOTIC RESISTANCE

The dramatic increase in antibiotic resistance worldwide has hampered the rate of successful eradication treatments[39,40].

Analysing trends in antibiotic resistance, the international prospective European Registry on *Helicobacter pylori* Management (Hp-EuReg) collected data from 3,974 patients who underwent susceptibility testing between 2013 and 2020. Comparing the periods from 2013 to 2016 and 2017 to 2020, a significant decrease in metronidazole resistance was observed (39% *vs.* 18%). Also, other frequently used antibiotics were reported with decreasing resistance rates. In 52% of therapy naïve patients tested during the whole period, resistances were detected[41]. A multicentric study in 24 centres from 18 European countries analysed the relationship between antibiotic consumption at community level with *H. pylori* resistance[40]. Overall, clarithromycin resistance was found in 21.4% of 1,211 subjects tested accompanied by resistance to levofloxacin in 15.8% and to metronidazole in 38.9%. Antibiotic consumption was significantly associated with these findings for the use of macrolides and also the consumption of quinolones on the community level. Another study from China confirmed the correlation between antibiotic use and resistance rates at the hospital level. These results emphasize the positive association of antibiotic consumption in general and increasing *H. pylori* resistance rates and highlight the need for antibiotic susceptibility testing (AST) before prescribing quinolones and macrolides for *H. pylori* eradication[42]. A systemic review and meta-analysis performed in the USA underlined the dramatic increase in metronidazole, levofloxacin, and clarithromycin resistance rates with each of these being higher than 30%[43].

Other data were generated in a study in Colombia in which AST was carried out using PCR on strains obtained from both a region with high gastric cancer risk and another with low gastric cancer risk. None of the strains showed resistance against amoxicillin, clarithromycin or rifampicin. Resistance rates for levofloxacin were high in both regions and metronidazole resistance was significantly higher in the low gastric cancer risk region[44]. The relevance of a regionally different prevalence of specific resistance genes was shown in studies from China (Shanghai and Wenzhou)[45-46] and India[47]. There were controversial results regarding the low proportion of amoxicillin resistant strains. Interestingly, the rate of amoxicillin resistance decreases significantly when samples are frozen prior to processing. Han et al[48] therefore systematically analysed amoxicillin resistant strains three months after freezing. In addition to phenotypic resistance, transcriptomic analyses were performed. It was demonstrated that cryopreservation leads to a downregulation of genes involved in both membrane structure and transport function. This might be a reason for the underestimation of amoxicillin resistance in general.

With respect to antibiotic stewardship approaches and the urgent need for new antibiotics to treat *H. pylori* infection, AST even prior to first-line treatment has come into focus.

The evolution of AST has led to the development of more and more genotypic approaches using different samples[49]. While phenotypic tests based on culturing methods used to represent the standard of care, new molecular-based genotypic diagnostic tests using fresh or even paraffin embedded biopsies or faecal samples have demonstrated promising results[50]. The use of faecal samples also enables AST in patients without indication for endoscopy. A group in Bulgaria demonstrated the utility of RT-PCR for the detection of *H. pylori* infection in parallel with the identification of clarithromycin resistance genes in faecal specimens. *H. pylori* status in this study was confirmed by urea breath test[51]. The accuracy of molecular methods for the detection of resistance varies between different, frequently used antibiotics. However, several studies confirmed a correlation between mutations of resistance genes and phenotypic resistance for clarithromycin and levofloxacin comparing *H. pylori* culture, Epsilometer test (E-Test) and Next Generation Sequencing (NGS)[52]. There have been further studies addressing the clinical reliability and comparability of different test modalities[53].

Host factors should not be forgotten in the discussion of this topic. In a meta-analysis on the relevance of host genetic variants associated with *H. pylori* eradication failure, Shah et al[54] analysed data from of 57 studies from 11 countries. Most of the studies included in this analysis focused on polymorphisms in the CYP2C19 gene which is relevant for metabolisation of the drugs used for eradication, with some of these being clearly associated with a higher likelihood of eradication failure.

Declining rates of eradication success led to recommendations by experts on further empiric treatment regimens as well as treatment guided by AST[55]. The expansion of materials used for the detection of antibiotic resistance to stools now allows antibiotic susceptibility guided therapies in patients without indication for endoscopy[56].

CONCLUSIONS

While many key pathways involved in the pathogenesis of *H. pylori* associated disease have been unravelled over the past years, there are still some areas that remain to be fully elucidated. The past year brought forth new data on the interaction of bacterial OMPs with epithelial surface adhesion factors, as well as a better understanding of TLR and NFκB dependent activation of immune cells and further pro-inflammatory processes. Genetic studies on both host and bacterial polymorphisms still present relevant new insights, but the number of publications in this field has decreased. This is in contrast to studies on the impact of *H. pylori* on the gastrointestinal microbiota and on mechanisms involved in *H. pylori* resistance to antibiotic treatment. Huge efforts are still spent on deciphering *H. pylori* induced gastric carcinogenesis as well as the impact of the bacteria and their pro-inflammatory effect on extragastric diseases. In all of these fields, relevant puzzle pieces have been discovered in the past year that will further help to see the full picture of the impact of *H. pylori* on human health.

Conflict of Interest

There is no conflict of interest for any of the authors with regards to the content of this manuscript.

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Informed Consent

Not applicable.

Authors Contribution

SF performed literature research and was responsible for the sections *H. pylori* and Gastritis and *H.pylori* and impact on other Systems. CS was responsible for the sections *H. pylori* and Microbiota and Antibiotic resistance. JB performed additional literature research and wrote the section *H.pylor*i and gastric carcinogenesis. All of the authors checked the manuscript and commented on all section.

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