Chapter

Pathophysiology of *H. pylori*

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Abstract

Helicobacter species were known for long as a causative agent of gastritis. *H. pylori* associated gastritis is characterized by the presence of acute and chronic inflammation. Previously, it was believed that in *H. pylori* gastritis, fundic inflammation was less important than that of the antral mucosa. However, *H. pylori* and gastroesophageal reflux disease create, or arise concurrently, may also be caused by the anatomical role of the inflammatory cell infiltrate. The source of *H. pylori* is mostly unknown. *H. pylori* has a small host range and is present in people and some non-human primates nearly exclusively. In rare cases, the presence of pets may be a concern for *H. pylori* infection; hence, pets should be isolated. There is also no definitive proof for zoonotic *H. pylori* transmission. The direct transmission from person to person, either oral or fecal-oral route or both, is expected to lead to new infections. *H. pylori* colonization is not an infection itself, but it impacts the relative likelihood that multiple pathological conditions of the upper gastrointestinal tract and even the hepatobiliary tract will grow. Therefore, *H. pylori* examination alone is not relevant but can be done in order to ascertain the cause of a basic disorder, such as peptic ulcer disease or to avoid disease, for example in subjects with family gastric carcinoma. A positive test result will validate the procedure, and a negative test result can suggest that other etiological causes or prevention steps needs to be examined. Gastritis is divided into acute and chronic. Several virulence factors play a role in the disease such as *cag* PAI (Pathogenicity Island) and VacA vacuolating cytotoxin. Different adhesins and their receptors aid in *H. pylori* colonization and invasion. Based on analogy with other mucosal infections, it was initially assumed that a protective immune response against *H. pylori* would predominantly be mediated by antibodies. Subsequent experiments have indicated that the relevance of the humoral system for protective immunity is only marginal. Antibodies can effectively prevent infection and reduce colonization in animal models.

Keywords: Helicobacter, Gastritis, CAG pathogenicity island, Vac A vaculating cytototoxin, Autoantibodies

1. Introduction

1.1 History of gastritis

Warren and Marshall in 1983 first recorded *H. pylori's* relationship of gastric mucosa in adults with antral gastritis [1]. Shortly thereafter, Hill *et al.,* four children who were afflicted with *H. pylori* had identified chronic mononuclear cell gastritis [2]. That same year, Cadranel and colleagues described organisms present in eight children with chronic, lymphocytic gastritis [3]. Subsequently, Drumm *et al.* observed *Helicobacter-like* organisms in 70% of 67 pediatric patients with a chronic-active

Gastritis [4]. Related findings have been made of spiral-shaped species colonizing the mucosa and the overlying gastric-epithelium mucus layer Infiltrate gastric inflammatory cells Czinn and Carrl in 25 children. More studies indicate that *H. pylori* colonization in the gastritis of a primarily chronic inflammatory cell infiltrate is almost always linked to gastritis in children [5, 6]. Reports of *H. pylori* eradication from gastric mucus suggest that the antral gastritis is resolved in combination with a single core case sequence. However, *H. pylori*-infected children have not undergone multicenter randomized controlled eradication trials and are important [6].

Studies in adults established the presence of the organism in nearly all cases of chronic gastritis [7]. At first, *H. pylori* was proposed to colonize inflamed tissue, rather than to induce inflammation, since gastritis is widespread in adults [7]. However, the prevalence of gastritis is less frequent in children thereby enabling the investigation of *H. pylori* as a cause for gastritis rather than an opportunistic colonizer of inflamed tissue [8]. Studies have observed that colonization of *H. pylori* in children with secondary causes, such as NSAID, eosinophilic gastroenteritis and Crohn's disease is not normal in the gastric mucosa [8]. These findings together show clearly the pathogenic role of *H. pylori* in the development of chronic antral gastritis in infants.

For over a century, bacteria have been known to be found in the human body [9]. These bacteria, however, were thought to be contaminants from digested food rather than true gastric colonizers. Around 20 years ago, the isolation and culture of a bacterial spiral species known later as *Helicobacter pylori* was announced successfully by Barry and Robin Warren [10], from the human stomach. Self-ingestion experiments by Marshall [11] and Morris [12] and later experiments with volunteers [13] demonstrated that these bacteria can colonize the human stomach, thereby inducing inflammation of the gastric mucosa. After ingestion of *H. pylori*, Marshall produced intermittent gastritis; Morris' condition progressed into more persistent gastritis, which cleared doxycycline and sub-salicylate bismuth after sequential care. These initial data were closely used as a stimulus in further studies, demonstrating that gastrointestinal disorders such as chronic gastric gastritis, peptic ulcer, lymphoma associated with gastric mucous membrane and stomach cancer can lead to a variety of upper gastrointestinal disorders. This knowledge has a direct therapeutic influence on disease control. In addition, insights into the pathogenesis of chronic disease are provided by the persistence of a pathogen in an area long believed to be sterile. This discovery has resulted in Robin Warren and Barry Marshall's "discovery of the bacterium *Helicobacter pylori* and his role in gastritis and peptic ulcer diseases" won the 2005 Nobel Prize in physiology or medicine.

The genus *Helicobacter* belongs to the subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella*, *Flexispira*, *Sulfurimonas*, *Thiomicrospira*, and *Thiovulum*. The Helicobacter genus consists of more than 20 species, all of which have been recognized officially. Members of the Helicobacter family are all microaerophile organisms and most of them are positive for catalase and oxidase and many but not all species also are positive for urease [14].

It is possible to separate Helicobacter species into two main lines, the gastric helicobacter species and the enterohepatic (nongastric) species of Helicobacter. They have a strong degree of organ specificity, which in general indicates gastric helicobacter cannot colonize the intestine or liver, and vice versa.

2. Gastric *Helicobacter* species

Gastric Helicobacter organisms have evolved to the unfriendly environments at the stomach surface and are currently suspected to colonize the stomachs of all

mammals through Helicobacter members. The urease is positive and extremely mobile by flagella are all recognized gastric Helicobacter species [15, 16]. Urease is believed to enable brief survival in a very acidic gastric lumen, but motility has been thought to allow quick travel into the more neutral pH of gastric mucosa; this may explain why the colonization of gastric mucosa is conditional upon both factors [17]. When joining, the Helicobacter gastric species display a chemical motility of urea and bicarbonate to the mucus layer [16]. The spiral morphology and flagellate motility help the viscous mucus layer penetrate, where the more pH-neutral conditions allow the genital Helicobacter species to grow.

i. *Helicobacter felis*

ii. *Helicobacter mustelae*

iii. *Helicobacter acinonychis*

iv. *Helicobacter heilmannii*.

H. pylori is a demanding micro-organism that needs complex media for development. Sometimes, blood or serum was applied to these medias. These supplements are additional food sources and can also be used for defending against long-chain fatty acid toxic effects [18].

H. pylori associated gastritis is characterized by the presence of acute and chronic inflammation, with immature surface epithelial cells [19]. Mucus degeneration is also found by successful cell renewal of epithelial cells. The degree of mucosal infection ranges from the lowest inflammatory infiltration in lamina propria to the extreme gastritis with thick mucoal inflammation with retained architecture. In extreme cases, all surface epithelium and gastric wells can be used as microabscesses for intraepithelial neutrophils [20].

Previously, it was believed that in *H. pylori* gastritis, fundic inflammation was less important than that of the antral mucosa [21]. However, *H. pylori* and gastroesophageal reflux disease create, or arise concurrently, may also be caused by the anatomical role of the inflammatory cell infiltrate [22]. Moreover, patients who have been receiving a proton pump inhibitor for acid suppression frequently have colonization of fundic and cardia mucosa by *H. pylori*. Carditis, of both a chronic and active phenotype, is frequent in *H. pylori*-infected adults [23]. Children require research to help establish the association between *H. pylori* infection, gastric inflammation sites and sequalae of long-term diseases.

H. pylori-associated gastritis in children is commonly not apparent at endoscopy, thereby making biopsy essential for definitive diagnosis [24]. Nodularity of the antral mucosa has been described in association with *H. pylori* gastritis in children [25]. Its value has not yet been identified. However, antral nodularity was found in *H. pylori* infected adults and less common in children [26].

Columbia or Brucella agar, (lysed) horse or sheep blood agar, or fetal calf serum as substitute, is widely used as a solid medium for regular isolation and *H. pylori* culture.

3. Transmission and sources of infection

Most unknown are the precise processes by which *H. pylori* is obtained. *H. pylori* has a small host range and is present in people and some non-human primates nearly exclusively.

In rare cases, the presence of pets may be a concern for *H. pylori* infection; hence, pets should be isolated [27]. There is also no definitive proof for zoonotic *H. pylori* transmission [28]. The direct transmission from person to person, either oral or fecal-oral route or both, is expected to lead to new infections. In saliva, vomit, gastric refluxate and diarrhea, *H. pylori* has been detected [29]. Yet there is no definitive proof that either of these products had the predominant transmission. This may be because most transmission research has concentrated on adults.

It indicates that for dentists, gastroenterologist, nurses, spousal partners or physicians with sexually transmitted diseases there was no clear rise in risk of carrying *H. pylori* [30]. As a result of these and other investigations, it is generally believed that acquisition mostly occurs in early childhood, most likely from close family members [31].

Crowding inside and outside families with children are both linked to the occurrence of *H. pylori* [32], Although the adult crowd seems less significant, except for some situations, for example amongst military recruits [33]. Several experiments have shown that *H. pylori* DNA is found in environmental bodies of water [34].

Spread via fecal contaminants is supported by the occurrence of *H. pylori* infections among institutionalized young people during outbreaks of gastroenteritis [35]. Additional sources are infected food, as *H. pylori* can briefly live on cooled food [36]. In tandem with *H. pylori's* intense exposure to oxygen demand, nutrient exclusion and temperatures outside 34–40°C [37]. The most likely path of direct person-to-person transmission.

The incidence of gastric cancer is higher in poor areas and in developing and advanced countries in lower socioeconomic groups [38]. Gastric cancer remains the most prevalent malignancy among men and the second most commonly identified among women in many countries of Latin America and Asia. Colombia and Japan recorded incidence rates of up to 80 per 100,000 populations. The gastric cancer in the United States and Western Europe, by comparison, affects less than 10 per 100,000 individuals per year [39]. However, ethnic groups with elevated risk remain in low-risk nations. For instance, in the US, gastric cancer is nearly double that prevalent among Blacks, Asians and Hispanics. It is noteworthy that *H. pylori* prevalence rates are 2-l0 times higher in all of these populations than in the total population [40].

4. Clinical aspects of *H. pylori*-associated diseases

H. pylori colonization is not an infection itself, but it impacts the relative likelihood that multiple pathological conditions of the upper gastrointestinal tract and even the hepatobiliary tract will grow. Therefore, *H. pylori* examination alone is not relevant but can be done in order to ascertain the cause of a basic disorder, such as peptic ulcer disease or to avoid disease, for example in subjects with family gastric carcinoma. A positive test result will validate the procedure, and a negative test result can suggest that other etiological causes or prevention steps needs to be examined.

4.1 Types of Disease

Although gastric *H. pylori* colonization causes histologic gastritis in all infected persons, only a minority experience any apparent clinical symptoms. *H. pylori*-positive patients are estimated to have a 10–20 percent life-cycle risk for ulcer and a 1–2 percent risk for distal gastric cancer [41]. The probability of developing *H. pylori* disorders depends on a range of bacterial, host, and environmental factors, most of which are related to gastritis pattern and severity (**Figure 1**).

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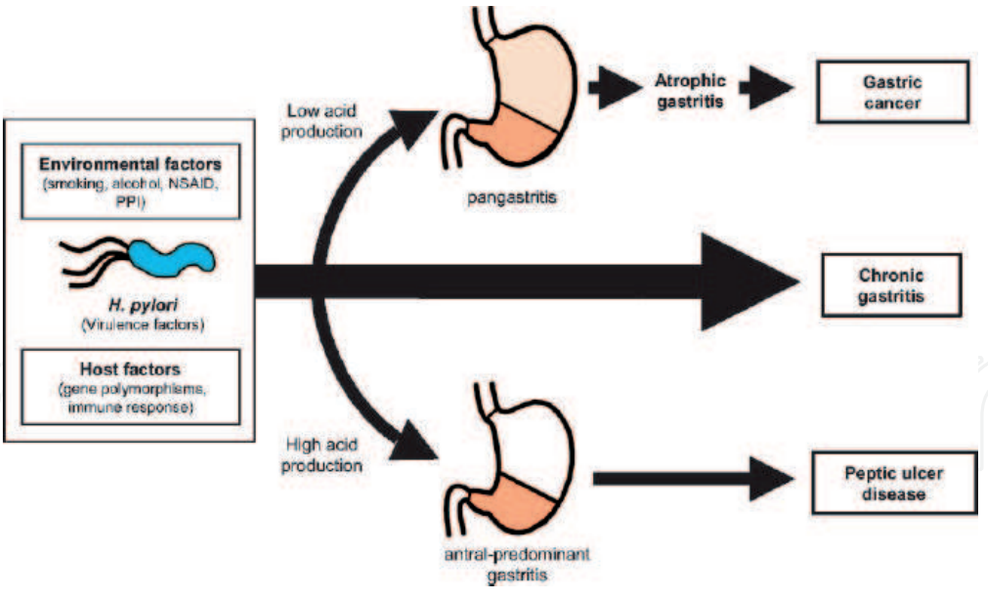


Figure 1.
Schematic representation of the factors contributing to gastric pathology and disease outcome in H. pylori *infection [42].*

4.2 Acute and chronic gastritis

The scale of *H. pylori* colonization nearly always contributes to gastric mucosa invasion of neutrophilic and mononuclear cells in the antrum and corpus. The principal condition of this chronic active gastritis is *H. pylori* colonization, in particular as consequence of this chronic inflammation phase, and other *H. pylori*-related conditions [42].

4.2.1 Acute gastritis

Acute infection results are uncommon and come mostly from accounts of subjects who knowingly or accidentally took *H. pylori* or were exposed to hazardous substance procedures [43]. Recently, an *H. pylori* infection human challenge model was introduced, which permitted managed acute infection analysis with deliberate safe volunteer infection by a well-characterized *H. pylori* laboratory strain [44]. Along with these findings, these reports have shown that the acute process of *H. pylori* colonization can include temporary non-specific dyspeptic symptoms, including completeness, nausea, vomiting, and severe inflammation of the proximal, and distal stomach mucus or pangastritis. This process is also related to the period of months of hypochlorhydria. It is uncertain whether spontaneous clearing and resolution of gastritis can be accompanied by this initial colonization and, if so, how often that happens. Further trials in young children with serology or breathing tests have shown that certain patients in this age group might spontaneously lose the infection [45]; This was not found in the development of atrophic gastritis other than under particular circumstances.

Studies of homozygotic twins however demonstrated a concordance with their classification as *H. pylori* regardless of their cohabitation or break [46]. This consensus between heterozygous twins has not been observed. This indicates that some people are likely to be colonized with *H. pylori* while others may avoid or eradicate a proven infection. This theory is also backed by the finding that *H. pylori* sensitivity in many developing countries is very strong in young age and yet chronic *H. pylori* infections are never acquired by any individuals.

4.2.2 Chronic gastritis

When colonization becomes persistent, the acid secretion level and the gastritis distribution interact closely (**Figure 2**). The association between acid and bacterial growth arises from the counteractive effects of acid against bacterial growth and subsequent mutation inflammation on the acid separation and regulation. The effect of *H. pylori* infection is important in this relationship. *H. pylori* particularly colonizes the gastric antrum in subjects with intact acid secretion, where there are only a few parietal acid secretory cells present [42].

This pattern of colonization is linked to gastritis which prevails. Histological examination of the gastric corpus specimens shows that the amount of superficially colonized *H. pylori* bacteria is reduced by chronically dormant inflammation and that. Those of which the secretion of acid is affected by some process have a more equal distribution of bacteria in the antrum and corpus and are in closer proximity of the mucosa bacteria, inducing pangastritis, which are predominate [47].

The acid secretion may be diminished by loss of parietal cells due to atrophic gastritis, but it can also happen when acid's secretive potential is intact, although the work of parietal cells is inhibited by vagotomy or acid-suppressive drugs, particularly proton pump inhibitors (PPIs) [47]. The subsequent active inflammation of the corpses raises hypochlorhydra parallel to an acute period of infection with a strong suppressive effect on the celestial function, since local inflammatory factors, including cytokines, like interleukin-1 beta (IL-1 β) are strongly suppressive. Different findings illustrate that. The first argument is that *H. pylori*-corpus gastritis frequently is related to hypochlorhydrate, and eradication treatment leads to greater secretion of acid [48].

Secondly, *H. pylori* corpus gastritis augments the acid-suppressive effects of PPIs [49]. As a result, *H. pylori*-positive patients with gastroesophageal reflux disease (GERD) may respond somewhat faster to PPI treatment both with respect to symptom resolution and with healing of esophagitis [50], However, this effect of everyday clinical practice is marginal and essentially negligible. This means that the status of *H. pylori* is not general in decision-making regarding GERD dose of PPI medication. A third observation in favor of the acid repression effects of active corpus gastritis has been made in more recent significant research that indicates the risk of corpus predominant pangastritis from subject with proinflammatory genotype predisposing people to atrophic gastritis, intestinal metaplasia and gastric cancer [51].

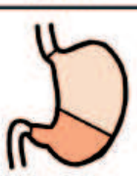
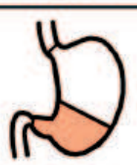
Pattern of gastritis	Gastric histology	Duodenal histology	Acid secretion	Clinical condition
 Pan-gastritis	• Chronic inflammation • Atrophy • Intestinal metaplasia	• Normal	• Reduced	• Gastric ulcer • Gastric cancer
 Antral-predominant	• Chronic inflammation • Polymorph activity	• Gastric metaplasia • Active chronic inflammation	• Increased	• Duodenal ulcer

Figure 2.
Acid secretion and the associated pattern of gastritis play an important role in disease outcome in H. pylori *infection. The figure displays the correlations between the pattern of* H. pylori *colonization, inflammation, acid secretion, gastric and duodenal histology, and clinical outcome [42].*

Although colonization by *H. pylori* is almost invariably associated with gastritis, and gastritis is mainly attributed to colonization by *H. pylori*, gastritis is due to other causes of gastritis, such as cytomegalovirus, chronic inflammatory idiopathic diseases, and auto-immune disease such as Crohn's disease and pernicious anemia.

5. Role of *H. pylori* virulence factors

5.1 *cag* PAI (Pathogenicity Island)

Although the *H. pylori* infection nearly always triggers chronic active gastritis, most affected patients are free of apparent health signs and have no other complications [52]. This lead to the belief that some strains could be more virulent than others. Early studies of variations of *H. pylori* strains demonstrated the capacity of such virulent strains to cause morphological changes, vacuolizations and successive degeneration from in vitro-cultivated cells. This pathogenicity is linked [53]. This activity was then linked to the presence of a protein with a molecular mass of approximately 140 kDa that was named CagA (for "cytotoxinassociated gene A").

The CagA protein is a highly immunogenic protein encoded by the *cagA* gene [54]. About 50–70 percent of *H. pylori* strains have this gene [55] and is a marker for the presence of a genomic PAI of about 40 kb that, depending on the strain analyzed, encodes between 27 and 31 proteins [54]. Strains carrying the Cag PAI are called CagA+ strains, as their ability to cause major antibody titers against the CagA marker protein is widely recognized in patients. In CagA patients, inflammation is typically higher and the probability of developing a signs (peptic ulcer or gastric cancer) in western populations is considerably higher [56], though not in Asian populations [57]. While CagA+ strains are associated with a higher risk of ulceration, gastritis and gastric cancer, cag PAI strains are also associated with a higher risk of peptic ulcer or gastrointestinal cancer, even though at a smaller frequency.

Eighteen cag PAI-coded proteins are used to form a type IV secretive unit that forms a structure like a syringe that is able to penetrate gastric epithelial cells and to promote the translocation of CagA, peptidoglycan, and probably other bacterial components into host cells [58] (**Figure 3**). Once delivered inside the cell, the CagA protein is phosphorylated at tyrosine residues in EPIYA motifs [59] by Src family kinases [60]. Phosphorylated CagA interacted then with a number of host signaling molecules, including tyrosine phosphate SHP-2, which results in morphological changes in the epithelial cells [61].

Apoptosis of T cells is impaired by the cag PAI since the immune response is also affected [62]. The association of type IV formation with the host cell also results in pro-inflammatory cytokines in epithelial cell induction [63].

It was originally believed that this proinflammatory cytokines are caused by a CagA protein itself, but nowadays CagA only plays, if any, a minor role in triggering them [63]. It is possible that the intimate contact with the IV-type form contributes to peptidoglycan leak into the eukaryotic cell [64] (**Figure 3**), although it cannot be ruled out completely that the activation of the IL-8 signaling cascade results from the translocation of a thus-far-unknown bacterial factor [63].

Tyrosine Phosphorylation is necessary for binding CagA to SHP-2 within the CagA EPIYA motif. [65]. The number of EPIYA tyrosine phosphorylation motives within the CagA proteins of various *H. pylori* isolates varies considerably. CagA is specifically correlated with the amount of repetitions of tyrosine phosphorylation. [66]. Strains with a larger number of CagA repetitions cause more marked morphological changes in cultivated epithelial cells [67] and an increased risk of gastric carcinogenesis being correlated. CagA also interacts through SH2 domains with the

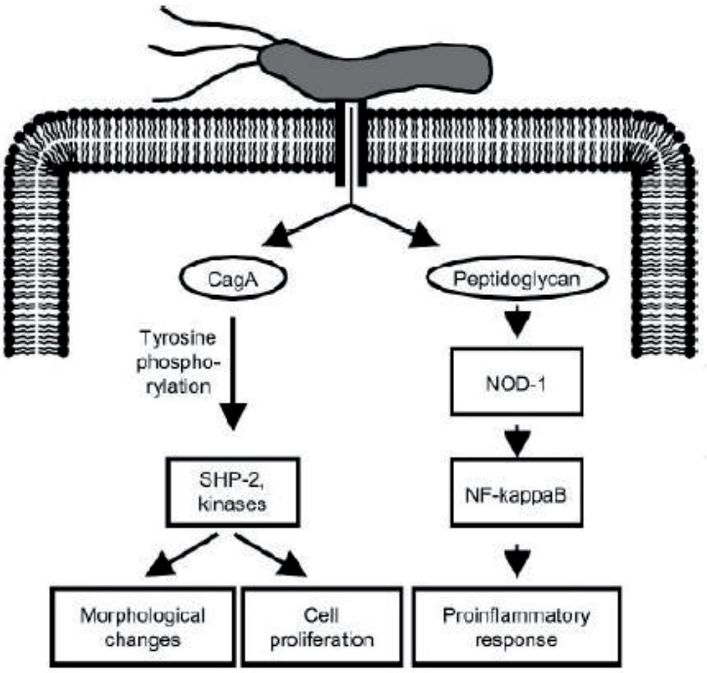


Figure 3.
Schematic representation of the different roles of the Cag type IV secretion system in immune modulation, cell proliferation, and morphological changes [42].

c-terminal Src kinase, which contributes to c-tyrosine Src's kinase inactivation. This inactivation, since it mediates CagA tyrosine phosphorylation, leads to a decrease in CagA phosphorylation, thereby creating a feedback loop to control CagA behavior [65]. This cross talk of host-pathogen results in tested virulence and can thus serve to colonize the host for a lifetime.

5.2 VacA vacuolating cytotoxin

Around 50% of all strains of *H. pylori* secrete VacA, a highly immunogenic 95 kDa protein that is causing massive vacuolisation of epithel cells in vitro [68]. VacA is a key factor in both peptic ulceration and gastric cancer pathogenesis. Though VacA is not necessary for in vitro growth of *H. pylori* the murine gastric colonization by *H. pylori* has been shown to make a substantial contribution [69].

The activities of VacA include development of the membrane channel, endosomal and lysosomal disorders, incorporate cell receptor signaling effects and cytoskeleton-related interference with cell-dependent functions, apoptosis induction and immune regulation (**Figure 4**). Although vacuolization is readily observed in vitro, it does not seem to occur *in vivo* [42]. The VacA protein is formed with a protoxin of 140 kDa and is broken into the shape of 95 kDa as it is secreted.

While all strains have a functional vacA gene, the vacuolating activities among strains differ considerably [69]. This is due to the sequence heterogeneity within the *vacA* gene at the signal region (s) and the middle region (m). The s region of the gene, which encodes the signal peptide, occurs as either an s1 or s2 type, whereas the m region, which contains the p58 cell binding domain, exists as an m1 or m2 type [70].

In the cell epithelial membrane, VacA forms pores that cause urea and anions to be released from the host cells. It also enhances transcellular penetration, resulting in nutrient and cation releases [71]. Interestingly, a major portion of the secreted toxin does not go to the environment, but is bound to the outer membrane of *H. pylori*. These toxin clusters are passed to the host cell surface following bacterial

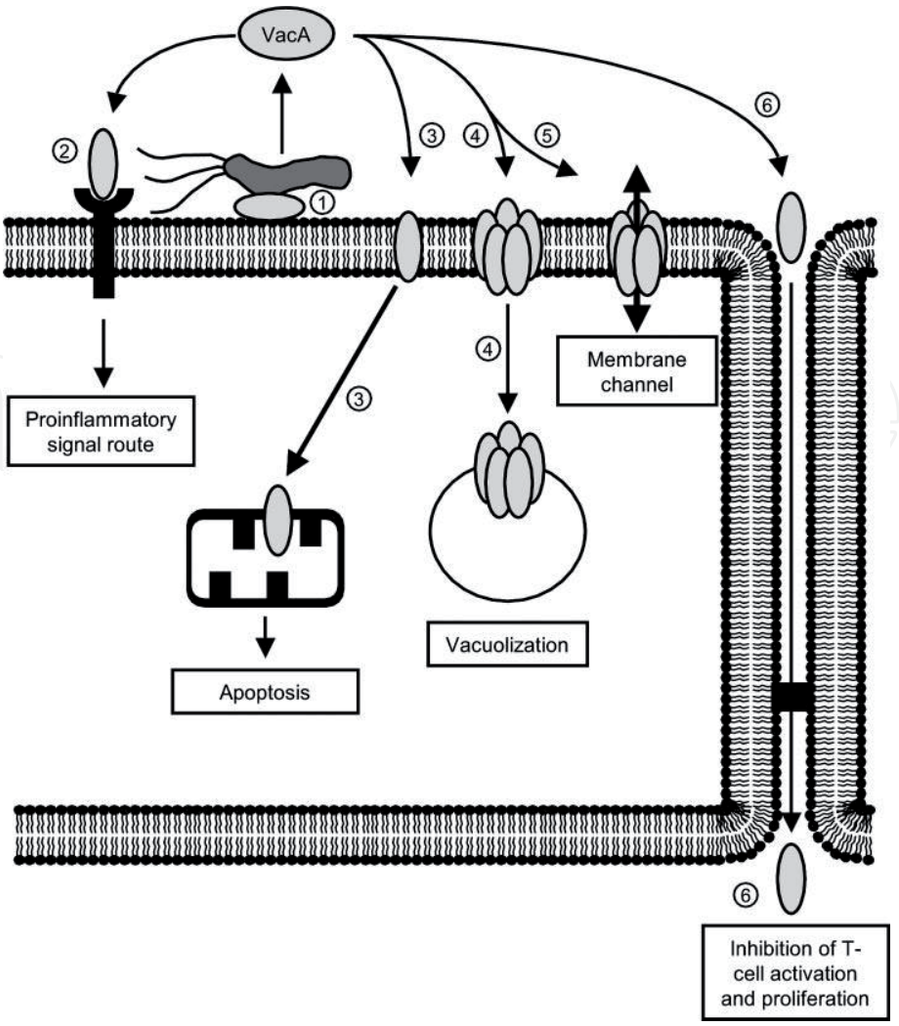


Figure 4.
The VacA protein influences cellular processes via different routes, thus assisting in chronic colonization of the gastric mucosa by H. pylori*. (1) Surface-bound VacA may be directly delivered to the cell membrane. Secreted VacA may either (2) bind to a cell membrane receptor and initiate a proinflammatory response, (3) be taken up directly by the cell and be trafficked to the mitochondria and induce apoptosis, (4) be taken up by pinocytosis and induce vacuolization, (5) form a membrane channel, resulting in leakage of nutrients to the extracellular space, or (6) pass through the tight junctions and inhibit T-cell activation and proliferation. (Modified with permission from* Nature Reviews Microbiology *[23].*

interaction with their host cells and have their toxic effects. This touch-dependent mechanism for direct delivery proposes the inclusion in bacterial-cell contact of particular receptors. However, such a receptor has not yet been identified [72].

Secreted VacA can be further processed into a 33-kDa N-terminal fragment and a 55-kDa C-terminal fragment through proteolytic cleavage. In the development of anion channels, the N-terminal protein plays an important role while C-terminal proteins mediate cell binding [73].

In spite of the proteolytic cleavage, these fragments remain noncovalently associated with each other [74]. Spontaneously purified VacA forms oligomeric aggregates and disassembles in the active monomers of pores in the cell membrane after exposed to acidic pH. Spontaneously purified VacA forms oligomeric aggregates and disassembles in the active monomers of pores in the cell membrane after exposed to acidic pH but are likely to be an *in vitro* artifact [75].

While several VacA-mediated effects are induced by membrane binding and pore formation, VacA also reaches the cytosol, then accumulates in the mitochondrial inner membrane and causes apoptosis, activating endogenous mitochondrial channels [76]. The proapoptotic influence of VacA is based on the cell type and may

be restricted to gastric epithelial cells like parietal cells. This may result in reduced acid secretion, thereby predisposing for development of gastric cancer [77].

6. Pathogenesis of infection

6.1 *H. pylori*-associated pathogenesis

Chronic active gastritis is the principal disease after *H. pylori* colonization. In all *H. pylori*-positive subjects this syndrome can be found. Various different factors such as colonizing stress characteristics, host physiology, immune response, diet, and development rate depend on the intragastric distribution and intensity of the chronic inflammatory process. Many of the complications of this chronic inflammation include *H. pylori*-induced ulcers, gastric cancer and lymphoma; in particular, ulcerative and gastric cancers arise in these people and in the areas of the most serious inflammation. Therefore, recognizing these factors is important in order to consider *H. pylori's* role in the etiology of the upper gastrointestinal disorder [42].

H. pylori colonizes the membrane in the stomach antrum of the gastric epithelia. *H. pylori* adhere to the stomach epithelia is a prime and significant step towards colonization of gastric mucosa and gastritis [78].

In duodenum, *H. pylori* only infects gastric mucosa and gastric metaplasia [79]. In comparison, on intestinal epithelium *H. pylori* is never seen. Dunn *et al*., stated in vitro, that *H. pylori* was more effective than human intestine (Int-407) cells, and sac cells with yolk bags, on human gastric epithelial cells [80]. Similarly, the adherence rate of *H. pylori* to gastric cell lines (KatoIII, MKN45) was shown to be significantly higher than that to Int-407 cells. These results indicate that *H. pylori* has specific binding activity to human gastric epithelial cells [81].

In a logarithmic point, *H. pylori* has strong nourishment with a spiral morphology. But spiral to coccoid conversion may be inducted by alkaline pH, rise in temperature, antibiotic therapy, aerobics or anaerobics, or prolonged incubation. The type of coccoid *H. pylori* is known as viable but not cultivable. *H. pylori* coccoid bind to both the stomach epithelial MKN45 cells and the spiral form. Although it is unknown whether the coccoid form has any role in the infection pathogenesis., Cole *et al*." The type of coccoid has been reported to bind badly to gastric epithelial cells. In contrast to spiral shape the form induces a short interleukin 8 (IL-8) chain. The coccoid shape, by comparison, was more frequent than the spiral of *H. pylori* induces cellular changes of pedestal formation [82].

6.2 Gastric environment at the site of infection

The human stomach has a medium luminal pH, with elevations until around pH 4 during meals due to nutrient buffering, when set to natural physiological acid secretion [83]. *H. pylori* originally occupied a more neutral niche at the gastric region with gastric acidity defense offered by the secretion of bicarbonate from epithelial cells and mucus. Studies with glass microelectrodes indicated a pH gradient in the stomach mucus and an epithelial pH virtually neutral [84]. The measuring technique may have delayed these studies as open tip microelectrodes may have stopped proton diffusion. Later microelectrode experiments using a similar method of calculation in mice found that all obstacles to the proton diffusion were eliminated and acidic pH was indicated in the bacterial niche [85]. Fluorescent dyeing tests in the anesthetic mice's externalized stomachs showed an acidic gastral pH surface regardless of the mucus layer [86]. The pH of the gastric surface is a

combination between the regulation of acid and alkaline secretion at a certain stage instead of the trapping of mucus-layer buffers or protons [87].

Analysis of *H. pylori* transcriptome provides additional evidence of acidic pH on gastric surfaces. Several in Vitro experiments have reported improvements in acidic pH expression of the gene using varying duration and conditions of incubation [87–89]. The unifying finding of these research studies is that there are a variety of genes that alter expression depending on environmental pH and indicate adaptation in order to permit gastric colonization.

The well-documented movements of *H. pylori* from its usual gastric niche to the fundus in human or gerbil acid inhibitory Therapy are proof that the bacteria need to remain in a particular pH setting [90–92]. The *H. pylori* transcriptome has been studied in the gerbil's stomach to correlate with in vitro pH changes [93]. Gerbil is a suitable model system since the gastric pH profile of *H. pylori* and its advanced sequelae are close to the ones found in humans [94–96]. The pattern of *H. pylori* gene changes in the gerbil stomach were comparable to gene changes seen in vitro at acidic pH, providing additional evidence for an acidic environment at the site of infection [97].

6.3 Attaching and effacement by adherence of *H. pylori*

Attaching and effacement is characterized by microvilli effacement, actin rearrangement and pedestal formation following bacterial adhesion to cells as described for enteropathogenic *Escherichia coli* (EPEC) [7]. The attachment and effacement of *H. pylori* in the gastric cells have been documented [97].

The tyrosine Phosphorylation of two host cell proteins (145 kDa and 105 kDa) has been shown to be inducted after *H. pylori* binding to gastric pathologic cells. Although it is hypothesized that tyrosine Phosphoryphorylation of host cell proteins is implicated in pathogenesis of gastric diseases related to *H. pylori* infection However, other researchers found that an attachment of *H. pylori* does not contribute to pedestal formation or actin rearrangement [98].

6.4 Adhesin of *H. pylori* and its receptor

H. pylori adhesively adheres to a receptor on the gastric cell surface by its adhesives. The adhesins and their receivers have been recorded in several respects. As the adhesion of *H. pylori* to cells is not entirely hindered by the human antibody to an adhesin, adherence by the use of many adhesins and their receptors is known to be the outcome.

6.5 HpaA (Sialyllactose-Binding Adhesin)

Evans *et al.* to purified 20 kDa protein as an adhesin of *H. pylori* recognizing N-acetylneuraminyllactose, and cloned its gene, *hpaA*. The protein HpaA functions as hemagglutinin and aggregates the fibrillary structure together. HpaA is stated to be a lipoprotein intracellular and the inactivation of the HpaA did not affect *H. pylori's* adherence to gastric cells. Consequently, the value of HpaA as *H. pylori* adhesin is contentious [99].

6.6 Adhesin recognizing phosphatidylethanolaurine

Specific binding of *H. pylori* to a glycerophospholipid species in the antrum of the human stomach was reported. The thin-layer chromatogram overlay technique showed this species to be a type of phosphatidylethanolamine. Since the exoenzyme from *Pseudomonas aeruginosa* displays similar binding specificity,

the binding of *H. pylori* to its lipid receptor was expected to be induced by an exoenzyme S-like adhesive [100].

6.7 BabA protein recognizing Lewisb antigen

The lewis antigens (Lewis, Lewisb, Lewisx, and LewisY) are one of the blood-group antigens that is flucosylated and expressed in human epithelics and erythrocytes. Lewis antigen that recognizes non-secretory blood groups (O) has been reported to be mediating adhesion of *H. pylori* to human gastric epithelial cells. Preferential relation between *H. pylori* and Lewisb antigen suggests that certain patients are more vulnerable to the development of peptic ulcers [101].

6.8 Adhesin recognizing extracellular matrix components

Many researchers have reported that *H. pylori* has been bound to different extracellular matrix components such as vitronectin, heparin sulfate, collagen, fibronectin, lactoferrin, plasminogen and laminin. There are about 20 Specific attachment of *H. pylori* to extracellular matrix components promotes bacterial colonization [102].

6.9 Induction of secretion of various cytokines from gastric cells

In vivo, *H. pylori* gastric infection induces several cytokines, including IL–l beta, IL–6, IL-7 and alpha tumor necrosis, to develop mucous membranes. IL-8 particularly consists of a small peptide (chemokine), secreted by a range of cell types, which helps to attract and activate neutrophils as a powerful inflammatory mediator. IL-8 development stimulation in gastric epithelial cells was recorded by *H. pylori*. The positive CagA strains displayed a significant increase in IL-8 relative to cag negative strains. Transposon inactivation of several genes in *cagA* pathogenicity island (PAl) showed that various genes *(cagB)* C) *D) E)* G) *H, ~ L) A1)* were responsible for the induction of IL-8 from gastric epithelial cells [29]. Recently, Li et al., [30] reported that multiple genes encoding HP052l, 0525, 0527, 0528 and 0529 ORF in the left half of the *cag* PAl of *H. pylori* are required for tyrosine-kinase dependent transcription of IL-8 in gastric epithelial cells. In addition, Yamaguchi et al., [31] reported the induction of IL-8 by HSP60 of *H. pylori* from gastric epithelial KatoIII cells [103].

7. *H. pylori* gastritis and the possible pathogenic

7.1 Role of anti-gastric autoimmune reactions

The serological study in *H. pylori* gastritis provided more indications on the relation between anti gastric autoimmunity and *H. pylori* gastritis. In older patients, *H. pylori* infection is linked to developing anti-parietal cell antibodies [55]. In addition, some *H. pylori*-infected patients, observed 32 years, produce both chronic atrophic gastritis and anti-parietal cell antibodies [104]. These patients eventually become *H. pylori* negative. Also Negrini *et al.,* reported on autoantibodies against gastric epithelial cells in up to 84% of *H. pylori* infected subjects [105].

When sera of *H. pylori-infected* subjects were screened for autoantibodies reacting against human gastric tissue by immunohistochemistry, for these autoantibodies two separate binding sites could be seen; The luminal membranes in the antral and corpus mucosa foveolar epithelial cells first and the channel membranes in the gastric corpus mucosa, second in the parietal cells. Antichannel autoanticorps were called the latter type [106].

7.2 Motility

In early childhood, *H. pylori* infection is obtained through oral-oral or oral-fecal infection. The microbe has to enter its chosen location of colonization, the mucosa of the gastric antrum in the first phase towards colonization. *H. pylori* has formed a spiral mode and unipolar scourge to enter the gastric niche to transit the mucus membrane that overlays the gastric epithelial surface. Host factors steer the migration of *H. pylori* towards the gastric mucosa by means of the chemical reaction of bacteria.

The transcription from intracellular localized components to extracellular flagellar filaments is transient regulated by the expression of the flagellar gene. *H. pylori* is primarily present on the usual acid-secreting stomach, with about one-third of the mucus layer next to the epithelial cells (0–5 μm) in a predominant 15–30 μm mucus above the antrum [107]. About 2 percent of bacteria bind to the gastric epithelium. In order to colonize this niche, the bacteria find that the host attractants or repellents and travel toward or away from them, respectively.

Urea, bicarbonate, pH, zinc, nickel, arginine, glutamine, histidine, and other amino acids elicit chemotactic responses by *H. pylori* [108–112]. These chemotactic factors are sensed by methylaccepting chemotaxis proteins (MCPs) that transduce the signal and alter flagellar rotation. *H. pylori* has at least four MCPs, the membrane proteins TlpA, TlpB, and TlpC and the cytoplasm located TlpD. TlpA senses arginine, other amino acids, and bicarbonate [108]; TlpB is required for pH and urea taxis and also senses the quorum sensing molecule autoinducer- 2 (AI-2) [113]; TlpC regulates whether acid is sensed as an attractant or repellent [111]; and TlpD senses the internal energy state of the bacterium [114].

7.3 Acid acclimation

Colonization is prevented by gastric acid. *H. pylori* is a neutrophil that rises from pH 6.0 to 8.0 and lives from pH 4.0 to 8.0. Since the median pH of the stomach is less than 2.0 and *H. pylori* not only lives in this high acidity, but also prospers, the single acid acclimation process has evolved. The ability of *H. pylori* to retain a near-neutral periplasmic pH in an acidic environment is accurate [115]. This is different from the acid resistance mechanism which enables a cytoplasmic pH near 5 to allow bacteria to transit the stomach [116]. Examples of proteins involved with acid resistance include the glutamate decarboxylase- glutamate aminobutyrate antiporter and the arginine decarboxylase-arginineagmatine antiporter, which consume protons and produce carbon dioxide, and the proton transporters including the F 1 F 0 ATPase and the Na +/2H + antiporter [117, 118]. These systems are designed to control the cytoplasm but do not monitor the pH of periplasm.

Gastric colonization is not possible if cytoplasmic pH cannot be elevated to a level that allows critical metabolic processes such as protein synthesis, a level of buffering that requires periplasmic pH regulation [116]. While *H. pylori* expresses some of the known acid resistance or tolerance genes [119], these proteins complement rather than explain gastric colonization. The principle component of acid acclimation is the neutral pH optimum, highly expressed cytoplasmic urease enzyme. The *H. pylori* urease gene cluster is made up of seven genes under the control of two promoters. *ureA* and *ureB*, under the control of the first promoter, encode the structural subunits of the urease enzyme [120].

Urease is a hexameric heterodimer that requires nickel incorporation for activation. Downstream from the second promoter are *ureI*, *ureE*, *ureF*, *ureG*, and *ureH* [121]. *ureI* encodes in an operon the only integral membrane protein. The Cytoplasmic proteins *UreE, UreF, UreG, and UreH* help to integrate nickel in apourease.

Urease is required for acid survival and gastric colonization [122, 123]. *H. pylori* urease production is constitutive, contributing about 10 percent of the total cell protein [124, 125]. A neutral pH-based cytoplasmic enzyme catalyzes the degradation of urea into carbonic acid and, eventually. With a low pH activity and inactivation, the pH-reduced into the region contained inside the intestines, the activity curve of the free urease is optimum near neutral. The activity of urease in intact bacteria is marginal at neutral pH and increases to no more than pH 6 to roughly pH 2.5 [126]. This curve of activity indicates a limit to urea entry to the enzyme. The only membrane protein of the urease gene cluster, UreI, was seen as a proton gated urea channel, which enables urea into cytoplasm at acidic pH [127]. Deletion of *ureI* leads to loss of acid activation of urease [125]. ureI deletion mutants cannot live in acid at physiologic urea concentrations. Periplasmic pH sinks as well as the medium pH drops. This leads to opening of UreI, movement of urea into the cytoplasm, and breakdown to the eventual end products of carbon dioxide and ammonia, catalyzed by the urease enzyme. The two gasses then buffer the periplasm to the pH range that is consistent with neutrophil survival without having to adjust the atmosphere with bulk pH.

Ni 2+ per active site are required for activation of urease, and a large fraction of urease can be inactive, especially at neutral pH [128, 129]. This will likely avoid the over-alkalization of this neutrophil in situations where the pH increases, and would thus create a urease pool that is primed and ready to go into action in setting a decrease in pH [130]. UreE forms a heterodimer with UreG and UreF with UreH, as evidenced by yeast two hybrid and homology analysis, and these protein pairs bind urease most likely via UreB to aid with nickel incorporation and enzyme activation [131, 132]. Each accessory protein has a specific role in urease activation. UreE aids directly with incorporation of nickel into the active site [132]. UreF prevents premature nickel binding [133]. UreG provides energy for assembly of urease. UreH provides stability for apourease [134]. A broad number of regulatory mechanisms, many of which are involved in acid survival, can be controlled directly and indirected by the nickel regulation protein NikR [135]. For example, NikR has been shown both in vitro and in vivo to positively regulate expression of *ureA* [136–139].

7.4 pH alteration and treatment efficacy

H. pylori is unique for survival in an acidic gastric environment, but bacteria are separated and formed at neutral pH as a neutrophil. Transcription of growth-dependent genes in higher medium pH is increased [140]. Most antibiotics used in treating *H. pylori* infection are bacterial-dependent for optimum effectiveness. Ampicillin is slightly more effective at near-neutral pH against *H. pylori* in vitro [140]. Adding bismuth to the treatment regimens also has a pH effect, at least in part, because the compound impairs the proton entry and reduces the decrease in cytoplasmic pH with medium acidification, which improves bacterial metabolism and increased antibiotic effectiveness [141]. With this in mind, The more bacteria are separated in the therapeutic cycle, the more successful conventional treatment, a proton pump inhibitor and a triple or quadruple therapy regimen of antibiotics are used. This concept is likely homologous to the concept of persisters seen across bacterial species.

Persisters are members of a bacterial population that survive exposure to bactericidal antibiotics yet, when re-cultured, display the same antibiotic sensitivity as the population as a whole [142, 143]. *H. pylori* that are not dividing at administered at antibiotic time will not be eliminated, leaving a limited population

of viable bacteria that can restore stomach colonization when antibiotics are stopped. At recommended doses, drugs currently available in acid blockade will not achieve the required sustained pH shift to imitate the bactericidal effect seen in in vitro studies [140, 144].

The current treatment effectiveness challenges can be solved by introducing non antibiotic treatment schemes, using the colonization mechanisms mentioned here, by prevention, intervention or acclimatization of motility, adhesion or acidity. The in vitro efficacy of the carbonic anhydrase inhibitor acetazolamide against *H. pylori* is one example of a potential treatment targeting acid acclimation and periplasmic pH regulation [123]. In the creation of new and better treatment regimes, a continuous research and understanding of the molecular processes of *H. pylori* gastric colonization are crucial.

8. Immune response role of antibodies in protective immunity

Analogy with other mucosal pathogens was originally thought to be primarily antibodies to mediate a defensive immune reaction against *H. pylori*. Subsequent studies found that the importance of the humoral immunity mechanism is negligible. Antibodies can prevent infection successfully and decrease the colonization in animal models studies [145]. *H. pylori* infection results in an induction of a Th1-polarized response that does not result, however, in clearance of the infection. This is striking, because the primary function in sterilizing Immunity is stated to be cellular rather than humoral immunity [146], Although it is now widely agreed that *H. pylori*-induced gastritis and/or pathology primarily rely on Th1 cells and Th1 cytokines [147]. While a polarized Th2 reaction defends against this pathology, it doesn't generally include the defense of Th2 cells after immunization. In fact, Th1-polarized T cells recruit mononuclear cells to the infection site instead of Th2-polarized, thereby removing bacteria [148].

9. Conclusion

From various studies it is concluded that gastritis still pose world -wide burden and requires extensive studies on the pathogenesis of the disease. *H. pylori* remains the most causative agent and it possess the virulence factors such as cag pathogenicity island and vacA vacuolating cytotoxin. These factors influence cellular processes via different routes, thus assisting in chronic colonization of the gastric mucosa by *H. pylori.* Gastritis infection highly results in induction of the protective immune response. Further studies should focus on the immunity to gastritis and the role of cytokines should be ruled out.

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References

[1] Warren. R., and Marshall B., 1983, Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet. 1:1273-1275.*

[2] Hill R., Pearman]., Worthy P, Caruso V, Goodwin S., and Blincow E., 1986, *Campylobacter pyloridis* and gastritis in children [letter]. *Lancet. 1(8477):387.*

[3] Cadranel S., Goossens H., De Boeck M., Malengreau A., Rodesch P, and Butzler P, 1986, *Campylobacter pyloridis* in children [letter]. *Lancet. 1(8483):735-736.*

[4] Drumm B., O'Brien A, Cutz E., and Sherman P, 1987, *Campylobacter pyloridis*-associated primary gastritis in children. *Pediatrics.* 80(2): 192-195.

[5] Kilbridge PM., Dahms B. B., and Czinn S, 1988, *Campylobacter pylori*-associated gastritis and peptic ulcer disease in children. *Am. Dis. Child.* 142(11): 1149-1152.

[6] Yeung C. K., Fu K. H., Yuen K. Y, *et al.,* 1990, *Helicobacter pylori* and associated duodenal ulcer. *Arch. Dis. Child.* 65(11): 12121216.

[7] Peterson W. L., 1991, *Helicobacter pylori* and peptic ulcer disease. *N. Engl. Med.* 324(15): 1043-1048.

[8] Drumm B., Sherman P, Cutz E., and Karmali M., 1987, Association of *Campylobacter pylori* on the gastric mucosa with antral gastritis in children. *N. Engl. Med. 316(25):1557-1561.*

[9] Bizzozero, G. 1893. Ueber die schlauchfö rmigen Drü sen des Magendarmkanals und die Beziehungen ihres Epithels zu dem Oberflä chenepithel der Schleimhaut. Dritte mitteilung. Archiv Mikroskopische Anat. 43:82-152.

[10] Warren, J. R., and B. J. Marshall. 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet i:1273-1275.

[11] Marshall, B. J., J. A. Armstrong, D. B. McGechie, and R. J. Glancy. 1985. Attempt to fulfil Koch's postulates for pyloric *Campylobacter*. Med. J. Austr. 142:436-439.

[12] Morris, A., and G. Nicholson. 1987. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. Am. J. Gastroenterol. *82*:192-199.

[13] Morris, A. J., M. R. Ali, G. I. Nicholson, G. I. Perez-Perez, and M. J. Blaser. 1991. Long-term follow-up of voluntary ingestion of Helicobacter pylori. Ann. Intern. Med. 114:662-663.

[14] Fox, J. G. 2002. The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. Gut 50:273-283.

[15] Solnick, J. V., and D. B. Schauer. 2001. Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. Clin. Microbiol. Rev. 14:59-97.

[16] Yoshiyama, H., and T. Nakazawa. 2000. Unique mechanism of *Helicobacter pylori* for colonizing the gastric mucus. Microbes Infect. 2:55-60.

[17] Schreiber, S., M. Konradt, C. Groll, P. Scheid, G. Hanauer, H. O. Werling, C. Josenhans, and S. Suerbaum. 2004. The spatial orientation of *Helicobacter pylori* in the gastric mucus. Proc. Natl. Acad. Sci. USA 101:5024-5029.

[18] Taneera, J., A. P. Moran, S. O. Hynes, H. O. Nilsson, W. Al-Soud, and T. Wadstrom. 2002. Influence of activated charcoal, porcine gastric mucin and beta-cyclodextrin on the morphology and growth of intestinal

and gastric Helicobacter spp. Microbiology 148:677-684.

[19] Bury-Mone, S., S. Skouloubris, C. Dauga, J. M. Thiberge, D. Dailidiene, D. E. Berg, A. Labigne, and H. De Reuse. 2003. Presence of active aliphatic amidases in *Helicobacter* species able to colonize the stomach. Infect. Immun. 71:5613-5622.

[20] Bruggraber, S. F., G. French, R. P. Thompson, and J. J. Powell. 2004. Selective and effective bactericidal activity of the cobalt (II) cation against *Helicobacter pylori*. Helicobacter 9: 422-428.

[21] Court, M., P. A. Robinson, M. F. Dixon, and J. E. Crabtree. 2002. Gastric *Helicobacter* species infection in murine and gerbil models: comparative analysis of effects of *H. pylori* and *H. felis* on gastric epithelial cell proliferation. J. Infect. Dis. 186:1348-1352.

[22] Cover, T. L. 1996. The vacuolating cytotoxin of *Helicobacter pylori*. Mol. Microbiol. 20:241-246.

[23] Cover, T. L., and S. R. Blanke. 2005. Helicobacter pylori VacA, a paradigm for toxin multifunctionality. Nat. Rev. Microbiol. 3:320-332.

[24] Contreras, M., J. M. Thiberge, M. A. Mandrand-Berthelot, and A. Labigne. 2003. Characterization of the roles of NikR, a nickel-responsive pleiotropic autoregulator of Helicobacter pylori. Mol. Microbiol. 49:947-963.

[25] Cover, T. L., and M. J. Blaser. 1992. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J. Biol. Chem. 267:10570-10575.

[26] Cover, T. L., U. S. Krishna, D. A. Israel, and R. M. Peek, Jr. 2003. Induction of gastric epithelial cell apoptosis by *Helicobacter pylori* vacuolating cytotoxin. Cancer Res. 63:951-957.

[27] Herbarth, O., P. Krumbiegel, G. J. Fritz, M. Richter, U. Schlink, D. M. Muller, and T. Richter. 2001. Helicobacter pylori prevalences and risk factors among school beginners in a German urban center and its rural county. Environ. Health Perspect. 109:573-577.

[28] Fox, J. G. 1995. Nonhuman reservoirs of *Helicobacter pylori*. Aliment. Pharmacol. Ther. 9(Suppl. 2):93-103.

[29] Sinha, S. K., B. Martin, B. D. Gold, Q. Song, M. Sargent, and C. N. Bernstein. 2004. The incidence of *Helicobacter pylori* acquisition in children of a Canadian First Nations community and the potential for parentto- child transmission. Helicobacter 9:59-68.

[30] Me'graud, F. 1995. Transmission of *Helicobacter pylori:* faecal-oral versus oral-oral. Aliment. Pharmacol. Ther. 9:85-91.

[31] van der Ende, A., E. A. J. Rauws, M. Feller, C. J. J. Mulder, G. N. J. Tytgat, and J. Dankert. 1996. Heterogeneous *Helicobacter pylori* isolates from members of a family with a history of peptic ulcer disease. Gastroenterology 111:638-647.

[32] Vincent, P., F. Gottrand, P. Pernes, M. O. Husson, M. Lecomtehoucke, D. Turck, and H. Leclerc. 1994. High prevalence of Helicobacter pylori infection in cohabiting children—epidemiology of a cluster, with special emphasis on molecular typing. Gut 35:313-316.

[33] Rowland, M., L. Daly, M. Vaughan, A. Higgins, B. Bourke, and B. Drumm. 2006. Age-specific incidence of Helicobacter pylori. Gastroenterology 130: 65-72.

[34] Queralt, N., R. Bartolome, and R. Araujo. 2005. Detection of *Helicobacter*

pylori DNA in human faeces and water with different levels of faecal pollution in the north-east of Spain. J. Appl. Microbiol. 98:889-895.

[35] Laporte, R., P. Pernes, P. Pronnier, F. Gottrand, and P. Vincent. 2004. Acquisition of *Helicobacter pylori* infection after outbreaks of gastro-enteritis: prospective cohort survey in institutionalised young people. BMJ 329: 204-205.

[36] Poms, R. E., and S. R. Tatini. 2001. Survival of Helicobacter pylori in ready-to-eat foods at 4 degrees C. Int. J. Food Microbiol. 63:281-286.

[37] Kusters, J. G., M. M. Gerrits, J. A. Van Strijp, and C. M. Vandenbroucke-Grauls. 1997. Coccoid forms of *Helicobacter pylori* are the morphologic manifestation of cell death. Infect. Immun. 65:3672-3679.

[38] Forman D., 1991, The etiology of gastric cancer. *fARC Sci. Publ. 105:22-32.*

[39] Hansson L. E., 2000, Risk of stomach cancer in patients with peptic ulcer disease. *World Surg.* 24(3):315-320.

[40] EI-Omar E. M., Oien K., Murray L. S., *et al.,* 2000, Increased prevalence of precancerous changes in relatives of gastric cancer patients: critical role of *H. pylori*. *Gastroenterology. 118(1):22-30.*

[41] Kuipers, E. J., J. C. Thijs, and H. P. Festen. 1995. The prevalence *of Helicobacter pylori* in peptic ulcer disease. Aliment. Pharmacol. Ther. 9(Suppl. 2):59-69.

[42] Kusters, J. G., Van Vliet, A. H., & Kuipers, E. J. (2006). Pathogenesis of *Helicobacter pylori* infection. *Clinical microbiology reviews*, *19*(3), 449-490.

[43] Sobala, G. M., J. E. Crabtree, M. F. Dixon, C. J. Schorah, J. D. Taylor, B. J. Rathbone, R. V. Heatley, and A. T. R. Axon. 1991. Acute *Helicobacter pylori* infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. Gut 32:1415-1418.

[44] Graham, D. Y., A. R. Opekun, M. S. Osato, H. M. El-Zimaity, C. K. Lee, Y. Yamaoka, W. A. Qureshi, M. Cadoz, and T. P. Monath. 2004. Challenge model for *Helicobacter pylori* infection in human volunteers. Gut 53:1235– 1243.

[45] Perez-Perez, G. I., R. B. Sack, R. Reid, M. Santosham, J. Croll, and M. J. Blaser. 2003. Transient and persistent *Helicobacter pylori* colonization in Native American children. J. Clin. Microbiol. 41:2401-2407.

[46] Malaty, H. M., L. Engstrand, N. L. Pedersen, and D. Y. Graham. 1994. *Helicobacter pylori* infection: genetic and environmental influences. A study of twins. Ann. Intern. Med. 120:982-986.

[47] Kuipers, E. J., A. M. Uyterlinde, A. S. Pena, H. J. Hazenberg, E. Bloemena, J. Lindeman, E. C. Klinkenberg-Knol, and S. G. Meuwissen. 1995. Increase of *Helicobacter pylori*-associated corpus gastritis during acid suppressive therapy: implications for long-term safety. Am. J. Gastroenterol. 90:1401– 1406.

[48] Ruiz, B., P. Correa, E. T. H. Fontham, and T. Ramakrishnan. 1996. Antral atrophy, Helicobacter pylori colonization, and gastric pH. Am. J. Clin. Pathol. 105:96-101

[49] Verdu', E., D. Armstrong, R. Fraser, F. Viani, J.-P. Idström, C. Cederberg, and A. L. Blum. 1995. Effect of *H. pylori* status on intragastric pH during treatment with omeprazole. Gut 36:539-543.

[50] Holtmann, G., C. Cain, and P. Malfertheiner. 1999. Gastric *Helicobacter pylori* infection accelerates healing of reflux esophagitis during treatment

with the proton pump inhibitor pantoprazole. Gastroenterology 117:11-16.

[51] El-Omar, E. M., M. Carrington, W. H. Chow, K. E. McColl, J. H. Bream, H. A. Young, J. Herrera, J. Lissowska, C. C. Yuan, N. Rothman, G. Lanyon, M. Martin, J. F. Fraumeni, Jr., and C. S. Rabkin. 2000. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404:398-402.

[52] Blaser, M. J., and J. C. Atherton. 2004. *Helicobacter pylori* persistence: biology and disease. J. Clin. Investig. 113:321-333.

[53] Leunk, R. D., P. T. Johnson, B. C. David, W. G. Kraft, and D. R. Morgan. 1988. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. J. Med. Microbiol. 26:93-99.

[54] Covacci, A., S. Censini, M. Bugnoli, R. Petracca, D. Burroni, G. Macchia, A. Massone, E. Papini, Z. Xiang, N. Figura, and R. Rappuoli. 1993. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA 90:5791-5795.

[55] Tummuru, M. K. R., T. L. Cover, and M. J. Blaser. 1993. Cloning and expression of a high molecular mass major antigen of *Helicobacter pylori:* evidence of linkage to cytotoxin production. Infect. Immun. 61:1799-1809.

[56] van Doorn, L. J., C. Figueiredo, R. Sanna, M. J. Blaser, and W. G. Quint. 1999. Distinct variants of *Helicobacter pylori cagA* are associated with *vacA* subtypes. J. Clin. Microbiol. 37:2306-2311.

[57] Peek, R. M., G. G. Miller, K. T. Tham, G. I. Pe´rez-Pe´rez, X. M. Zhao, J. C. Atherton, and M. J. Blaser. 1995. Heightened inflammatory response and cytokine expression *in vivo* to *cagA*_ *Helicobacter pylori* strains. Lab. Investig. 73:760-770.

[58] Segal, E. D., J. Cha, J. Lo, S. Falkow, and L. S. Tompkins. 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA. 96:14559-14564.

[59] Stein, M., R. Rappuoli, and A. Covacci. 2000. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after *cag*-driven host cell translocation. Proc. Natl. Acad. Sci. USA 97:1263-1268

[60] Stein, M., F. Bagnoli, R. Halenbeck, R. Rappuoli, W. J. Fantl, and A. Covacci. 2002. c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. Mol. Microbiol. 43:971-980.

[61] Selbach, M., S. Moese, S. Backert, P. R. Jungblut, and T. F. Meyer. 2004. The *Helicobacter pylori* CagA protein induces tyrosine dephosphorylation of ezrin. Proteomics 4:2961-2968.

[62] Smith, M. A., and D. I. Edwards. 1995. The influence of microaerophilia and anaerobiosis on metronidazole uptake in *Helicobacter pylori*. J. Antimicrob. Chemother. 36:453-461.

[63] Fischer, W., J. Puls, R. Buhrdorf, B. Gebert, S. Odenbreit, and R. Haas. 2001. Systematic mutagenesis of the *Helicobacter pylori* cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. Mol. Microbiol. 42:1337-1348.

[64] Viala, J., C. Chaput, I. G. Boneca, A. Cardona, S. E. Girardin, A. P. Moran, R. Athman, S. Memet, M. R. Huerre, A. J. Coyle, P. S. DiStefano, P. J. Sansonetti, A. Labigne, J. Bertin, D. J. Philpott, and R. L. Ferrero. 2004. Nod1 responds to

peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. Nat. Immunol. 5:1166-1174.

[65] Tsutsumi, R., H. Higashi, M. Higuchi, M. Okada, and M. Hatakeyama. 2003. Attenuation of *Helicobacter pylori* CagA SHP-2 signaling by interaction between CagA and C-terminal Src kinase. J. Biol. Chem. 278:3664-3670.

[66] Higashi, H., R. Tsutsumi, A. Fujita, S. Yamazaki, M. Asaka, T. Azuma, and M. Hatakeyama. 2002. Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc. Natl. Acad. Sci. USA 99:14428-14433.

[67] Yamazaki, S., A. Yamakawa, Y. Ito, M. Ohtani, H. Higashi, M. Hatakeyama, and T. Azuma. 2003. The CagA protein of *Helicobacter pylori* is translocated into epithelial cells and binds to SHP-2 in human gastric mucosa. J. Infect. Dis. 187:334-337.

[68] Salama, N. R., G. Otto, L. Tompkins, and S. Falkow. 2001. Vacuolating cytotoxin of *Helicobacter pylori* plays a role during colonization in a mouse model of infection. Infect. Immun. 69:730-736

[69] van Doorn, L. J., C. Figueiredo, R. Sanna, S. Pena, P. Midolo, E. K. Ng, J. C. Atherton, M. J. Blaser, and W. G. Quint. 1998. Expanding allelic diversity of *Helicobacter pylori vacA*. J. Clin. Microbiol. 36:2597-2603.

[70] Montecucco, C., and M. de Bernard. 2003. Molecular and cellular mechanisms of action of the vacuolating cytotoxin (VacA) and neutrophil-activating protein (HP NAP) virulence factors of *Helicobacter pylori*. Microbes Infect. 5:715-721.

[71] Fitchen, N., D. P. Letley, P. O'Shea, J. C. Atherton, P. Williams, and K. R. Hardie. 2005. All subtypes of the cytotoxin VacA adsorb to the surface of *Helicobacter pylori* post-secretion. J. Med. Microbiol. 54:621-630.

[72] Torres, V. J., S. E. Ivie, M. S. McClain, and T. L. Cover. 2005. Functional properties of the p33 and p55 domains of the *Helicobacter pylori* vacuolating cytotoxin. J. Biol. Chem. 280:21107-21114.

[73] Telford, J. L., P. Ghiara, M. Dell'Orco, M. Comanducci, D. Burroni, M. Bugnoli, M. F. Tecce, S. Censini, A. Covacci, Z. Xiang, et al. 1994. Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. J. Exp. Med. 179:1653-1658.

[74] Pelicic, V., J. M. Reyrat, L. Sartori, C. Pagliaccia, R. Rappuoli, J. L. Telford, C. Montecucco, and E. Papini. 1999. *Helicobacter pylori* VacA cytotoxin associated with the bacteria increases epithelial permeability independently of its vacuolating activity. Microbiology 145:2043-2050

[75] Willhite, D. C., and S. R. Blanke. 2004. *Helicobacter pylori* vacuolating cytotoxin enters cells, localizes to the mitochondria, and induces mitochondrial membrane permeability changes correlated to toxin channel activity. Cell. Microbiol. 6:143-154.

[76] Neu, B., P. Randlkofer, M. Neuhofer, P. Voland, A. Mayerhofer, M. Gerhard, W. Schepp, and C. Prinz. 2002. *Helicobacter pylori* induces apoptosis of rat gastric parietal cells. Am. J. Physiol. Gastrointest. Liver Physiol. 283:G309–G318.

[77] Testerman, T. L., McGee, D. J., & Mobley, H. L. (2001). Adherence and colonization. *Helicobacter pylori: physiology and genetics*, 379-417.

[78] Logan R. P. H., 1996, Adherence of *Helicobacter pylori*. *Aliment Pharmacal. Ther.* 10(Supp!. I): 3-15.

[79] Dunn B. E., Altmann M., and Campbell G. P., 1991, Adherence of *Helicobacter pylori* to gastric carcinoma cells: Analysis by flow cytometry. *Rev. lrifect. Dis.* 13(Supp!. 8):S657-S664.

[80] Yamamoto-Osaki T, Yamaguchi H., Taguchi H., Ogata S., and Kamiya S., 1995, Adherence of *Helicabacter pylori* to cultured human gastric carcinoma cells. *Ear. J Gastroenterol. Hepatol.* 7(Supp!.I): S89-S92.

[81] Cole S. P., Cirillo D., Kagnoff M. F., Guiney O. G., and Eckmann L., 1997, Coccoid and spiral *Helicobacter pylori* differ in their abilities to adhere to gastric epithelial cells and induce interleukin-8 secretion. *lrifect. Immun. 65:843-·846.*

[82] Teyssen S, Chari ST, Scheid J, Singer MV. Effect of repeated boluses of intravenous omeprazole and primed infusions of ranitidine on 24-hour intragastric pH in healthy human subjects. Dig Dis Sci. 1995;40:247-55.

[83] Code CF. Defense mechanisms of the gastric mucosa. Scand J Gastroenterol Suppl. 1981;67:201-4.

[84] Henriksnas J, Phillipson M, Storm M, Engstrand L, Soleimani M, Holm L. Impaired mucus-bicarbonate barrier in *Helicobacter pylori* -infected mice. Am J Physiol Gastrointest Liver Physiol. 2006;291:G396-403.

[85] Baumgartner HK, Montrose MH. Regulated alkali secretion acts in tandem with unstirred layers to regulate mouse gastric surface pH. Gastroenterology. 2004;126:774-83.

[86] McGowan CC, Necheva AS, Forsyth MH, Cover TL, Blaser MJ. Promoter analysis of *Helicobacter pylori* genes with enhanced expression at low pH. Mol Microbiol. 2003;48:1225-39.

[87] Merrell DS, Goodrich ML, Otto G, Tompkins LS, Falkow S. pH-regulated gene expression of the gastric pathogen *Helicobacter pylori*. Infect Immun. 2003;71:3529-39.

[88] Wen Y, Marcus EA, Matrubutham U, Gleeson MA, Scott DR, Sachs G. Acid-adaptive genes of *Helicobacter pylori*. Infect Immun. 2003;71:5921-39.

[89] Larkin CJ, Watson RGP, Sloan JM, Stevenson M, Ardill JE, Buchanan D. Distribution of atrophy in *Helicobacter pylori* -infected subjects taking proton pump inhibitors. Scand J Gastroenterol. 2000;35:578-82.

[90] Lee A, Dixon MF, Danon SJ, Kuipers E, Megraud F, Larsson H, et al. Local acid production and *Helicobacter pylori*: a unifying hypothesis of gastroduodenal disease. Eur J Gastroenterol Hepatol. 1995;7:461-5.

[91] Logan RP, Walker MM, Misiewicz JJ, Gummett PA, Karim QN, Baron JH. Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazole. Gut. 1995;36:12-6.

[92] Mollenhauer-Rektorschek M, Hanauer G, Sachs G, Melchers K. Expression of UreI is required for intragastric transit and colonization of gerbil gastric mucosa by *Helicobacter pylori*. Res Microbiol. 2002;153:659-66.

[93] Scott DR, Marcus EA, Wen Y, Oh J, Sachs G. Gene expression *in vivo* shows that *Helicobacter pylori* colonizes an acidic niche on the gastric surface. Proc Natl Acad Sci U S A. 2007;104:7235-40.

[94] Hirayama F, Takagi S, Kusuhara H, Iwao E, Yokoyama Y, Ikeda Y. Induction of gastric ulcer and intestinal metaplasia in mongolian gerbils infected with *Helicobacter pylori*. J Gastroenterol. 1996;31: 755-7.

[95] Matsumoto S, Washizuka Y, Matsumoto Y, Tawara S, Ikeda F, Yokota Y, et al. Induction of ulceration

and severe gastritis in Mongolian gerbil by *Helicobacter pylori* infection. J Med Microbiol. 1997;46:391-7.

[96] Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. *Helicobacter pylori* infection induces gastric cancer in mongolian gerbils. Gastroenterology. 1998;115: 642-8.

[97] Dytoc M., Gold B., Louie M., et aI., 1993, Comparison of *Helicobacter pylori* and attaching effacing Escherichia coli adhesion to eukaryotic cells. lrifect. Immun. 61:448-456.

[98] O'Toole P. W, Janzon L., Doig P., Huang., Kostrzynska M., and Trust T., 1995, The putative neuraminyllactose-binding hemagglutinin HpaA of *Helicabacter pylori* CCUG 17874 is a lipoprotein. *J Bacterial.* 177:6049-605 7.

[99] Lingwood C. A, Huesca M., and Kuksis A, 1992, The glycerolipid receptor for *Helicobacter pylori* (and exoenzyme S) is phosphatidyiethanolamine. *lrifect. Immun. 60:2470-2474.*

[100] Boren T, Falk P., Roth K. A., Larson G., and Normark S., 1993, Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science 262: 1892-1895.*

[101] Kondo I., Nagate T., Akashi T., Kaneda Y, Miyachi J., and Yamaguchi M., 1993, Presence of receptors for laminin, collagen, fibronectin and vitronectin on the cell surface of *Helicobacter pylori. Eur.* J *Gastroentero!. Hepatol.* 5:S63-S67.

[102] Yamaguchi H., Osaki T., Kurihara N., *et aI.,* 1999, Induction of secretion of interleukin-8 from human gastric epithelial cells by heat-shock protein 60 homologue of *Helieobaeter pylori.* J *Med. Microbiol. 48:927-933.*

[103] Uibo R., Vorobjova T, Metskula K., Kisand K., Wadstrom T, and Kivik T, 1995, Association of *Helicohacter pylori* and gastric autoimmunity: a population based study, *FEMS Immunol. Med. Microbiol.* 11:65.

[104] Negrini R., Lisato L., Zanella 1., Cavazzini L., Gullini S., Vilianacci v., Poiesi C., Albertini A., and Ghielmi S., 1991, *Helicobacter pylori* infection induces antibodies cross-reacting with human gastric mucosa, *Gastroenterology 101:437.*

[105] Faller G., Steininger H., Kranzlein., Maul H., Kerkau T, Hensen., Hahn E. G., and Kirchner T, 1997, Antigastric autoantibodies in *Helicobacter pylori* infection: implications of histological and clinical parameters of gastritis, *Gut 41:619.*

[106] Schreiber S, Konradt M, Groll C, Scheid P, Hanauer G, Werling HO, et al. The spatial orientation of *Helicobacter pylori* in the gastric mucus. Proc Natl Acad Sci U S A. 2004;101:5024-9.

[107] Cerda O, Rivas A, Toledo H. *Helicobacter pylori* strain ATCC700392 encodes a methyl-accepting chemotaxis receptor protein (MCP) for arginine and sodium bicarbonate. FEMS Microbiol Lett. 2003; 224:175-81.

[108] Foynes S, Dorrell N, Ward SJ, Stabler RA, McColm AA, Rycroft AN, et al. *Helicobacter pylori* possesses two CheY response regulators and a histidine kinase sensor, CheA, which are essential for chemotaxis and colonization of the gastric mucosa. Infect Immun. 2000;68:2016-23.

[109] Nakamura H, Yoshiyama H, Takeuchi H, Mizote T, Okita K, Nakazawa T. Urease plays an important role in the chemotactic motility of *Helicobacter pylori* in a viscous environment. Infect Immun. 1998;66:4832-7.

[110] Sanders L, Andermann TM, Ottemann KM. A supplemented soft agar chemotaxis assay demonstrates the

Helicobacter pylori chemotactic response to zinc and nickel. Microbiology. 2013;159(Pt 1):46-57.

[111] Worku ML, Karim QN, Spencer J, Sidebotham RL. Chemotactic response of *Helicobacter pylori* to human plasma and bile. J Med Microbiol. 2004;53(Pt 8):807-11.

[112] Rader BA, Wreden C, Hicks KG, Sweeney EG, Ottemann KM, Guillemin K. *Helicobacter pylori* perceives the quorum-sensing molecule AI-2 as a chemorepellent via the chemoreceptor TlpB. Microbiology. 2011;157(Pt 9):2445-55.

[113] Schweinitzer T, Mizote T, Ishikawa N, Dudnik A, Inatsu S, Schreiber S, et al. Functional characterization and mutagenesis of the proposed behavioral sensor TlpD of *Helicobacter pylori*. J Bacteriol. 2008;190:3244-55.

[114] Boren T, Falk P, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science. 1993;262:1892-5.

[115] Sakamoto S, Watanabe T, Tokumaru T, Takagi H, Nakazato H, Lloyd KO. Expression of Lewisa, Lewisb, Lewisx, Lewisy, siayl-Lewisa, and sialyl- Lewisx blood group antigens in human gastric carcinoma and in normal gastric tissue. Cancer Res. 1989;49:745-52.

[116] Gerhard M, Lehn N, Neumayer N, Boren T, Rad R, Schepp W, et al. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A. 1999; 96:12778-83.

[117] Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. 1998; 279:373-7.

[118] Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, et al. *Helicobacter pylori* SabA adhesion in persistent infection and chronic inflammation. Science. 2002;297:573-8.

[119] Ota H, Nakayama J, Momose M, Hayama M, Akamatsu T, Katsuyama T, et al. *Helicobacter pylori* infection produces reversible glycosylation changes to gastric mucins. Virchows Arch. 1998;433:419-26.

[120] Peck B, Ortkamp M, Diehl KD, Hundt E, Knapp B. Conservation, localization and expression of HopZ, a protein involved in adhesion of *Helicobacter pylori*. Nucleic Acids Res. 1999;27:3325-33.

[121] Odenbreit S, Faller G, Haas R. Role of the alpAB proteins and lipopolysaccharide in adhesion of *Helicobacter pylori* to human gastric tissue. Int J Med Microbiol. 2002;292:247-56.

[122] Marcus EA, Moshfegh AP, Sachs G, Scott DR. The periplasmic alpha-carbonic anhydrase activity of *Helicobacter pylori* is essential for acid acclimation. J Bacteriol. 2005;187:729-38.

[123] Ma Z, Gong S, Richard H, Tucker DL, Conway T, Foster JW. GadE (YhiE) activates glutamate decarboxylase- dependent acid resistance in *Escherichia coli* K-12. Mol Microbiol. 2003;49:1309-20.

[124] Booth IR. Regulation of cytoplasmic pH in bacteria. Microbiolo Rev. 1985;49:359-78.

[125] Padan E, Zilberstein D, Schuldiner S. pH homeostasis in bacteria. Biochim Biophys Acta. 1981;650:151-66.

[126] Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, *et al.* The complete genome sequence of the gastric

pathogen *Helicobacter pylori*. Nature. 1997;388:539-47.

[127] Akada JK, Shirai M, Takeuchi H, Tsuda M, Nakazawa T. Identifi cation of the urease operon in *Helicobacter pylori* and its control by mRNA decay in response to pH. Mol Microbiol. 2000;36: 1071-84.

[128] Eaton KA, Brooks CL, Morgan DR, Krakowka S. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. Infect Immun. 1991;59:2470-5.

[129] Eaton KA, Krakowka S. Effect of gastric pH on ureasedependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect Immun. 1994;62:3604-7.

[130] Hu LT, Mobley HL. Purifi cation and N-terminal analysis of urease from *Helicobacter pylori*. Infect Immun. 1990; 58:992-8.

[131] Mobley HL, Hu LT, Foxal PA. *Helicobacter pylori* urease: properties and role in pathogenesis. Scand J Gastroenterol Suppl. 1991;187:39-46.

[132] Scott DR, Marcus EA, Weeks DL, Lee A, Melchers K, Sachs G. Expression of the *Helicobacter pylori ureI* gene is required for acidic pH activation of cytoplasmic urease. Infect Immun. 2000;68:470-7.

[133] Weeks DL, Eskandari S, Scott DR, Sachs G. A H+-gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. Science. 2000; 287:482-5.

[134] Ha NC, Oh ST, Sung JY, Cha KA, Lee MH, Oh BH. Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. Nat Struct Biol. 2001;8:505-9.

[135] Scott DR, Marcus EA, Weeks DL, Sachs G. Mechanisms of acid resistance due to the urease system of *Helicobacter pylori*. Gastroenterology. 2002;123: 187-95.

[136] Ferrero RL, Cussac V, Courcoux P, Labigne A. Construction of isogenic urease-negative mutants of *Helicobacter pylori* by allelic exchange. J Bacteriol. 1992;174:4212-7.

[137] Heimer SR, Mobley HL. Interaction of *Proteus mirabilis* urease apoenzyme and accessory proteins identified with yeast two-hybrid technology. J Bacteriol. 2001;183:1423-33.

[138] Voland P, Weeks DL, Marcus EA, Prinz C, Sachs G, Scott D. Interactions among the seven *Helicobacter pylori* proteins encoded by the urease gene cluster. Am J Physiol Gastrointest Liver Physiol. 2003;284:G96-106.

[139] Moncrief MB, Hausinger RP. Purifi cation and activation properties of UreD-UreF-urease apoprotein complexes. J Bacteriol. 1996;178:5417-21.

[140] Moncrief MB, Hausinger RP. Characterization of UreG, identifi cation of a UreD-UreF-UreG complex, and evidence suggesting that a nucleotide-binding site in UreG is required for *in vivo* metallocenter assembly of *Klebsiella aerogenes* urease. J Bacteriol. 1997;179: 4081-6.

[141] Park IS, Carr MB, Hausinger RP. In vitro activation of urease apoprotein and role of UreD as a chaperone required for nickel metallocenter assembly. Proc Natl Acad Sci U S A. 1994;91:3233-7.

[142] van Vliet AH, Ernst FD, Kusters JG. NikR-mediated regulation of *Helicobacter pylori* acid adaptation. Trends Microbiol. 2004;12:489-94.

[143] Carpenter BM, West AL, Gancz H, Servetas SL, Pich OQ, Gilbreath JJ, et al. Crosstalk between the *Hp* ArsRS

two-component system and *Hp* NikR is necessary for maximal activation of urease transcription. Front Microbiol. 2015;6:558.

[144] Dosanjh NS, Hammerbacher NA, Michel SL. Characterization of the *Helicobacter pylori* NikR-*P* (*ureA*) DNA interaction: metal ion requirements and sequence specifi city. Biochemistry. 2007;46:2520-9.

[145] Dosanjh NS, West AL, Michel SL. *Helicobacter pylori* NikR's interaction with DNA: a two-tiered mode of recognition. Biochemistry. 2009;48:527-36.

[146] Evans SE, Michel SL. Dissecting the role of DNA sequence in *Helicobacter pylori* NikR/DNA recognition. Dalton Trans. 2012;41:7946-51.

[147] Mohammadi, M., S. Czinn, R. Redline, and J. Nedrud. 1996. Helicobacter specific cell-mediated immune responses display a predominant Th1 phenotype and promote a delayed-type hypersensitivity response in the stomachs of mice. J. Immunol. 156:4729-4738

[148] Sommer, F., H. Wilken, G. Faller, and M. Lohoff. 2004. Systemic Th1 immunization of mice against *Helicobacter pylori* infection with CpG oligodeoxynucleotides as adjuvants does not protect from infection but enhances gastritis. Infect. Immun. 72:1029-1035