

Review Article

The Human Gastric Pathogen *Helicobacter pylori* and Its Association with Gastric Cancer and Ulcer Disease

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With the momentous discovery in the 1980's that a bacterium, *Helicobacter pylori*, can cause peptic ulcer disease and gastric cancer, antibiotic therapies and prophylactic measures have been successful, only in part, in reducing the global burden of these diseases. To date, ~700,000 deaths worldwide are still attributable annually to gastric cancer alone. Here, we review *H. pylori*'s contribution to the epidemiology and histopathology of both gastric cancer and peptic ulcer disease. Furthermore, we examine the host-pathogen relationship and *H. pylori* biology in context of these diseases, focusing on strain differences, virulence factors (CagA and VacA), immune activation and the challenges posed by resistance to existing therapies. We consider also the important role of host-genetic variants, for example, in inflammatory response genes, in determining infection outcome and the role of *H. pylori* in other pathologies—some accepted, for example, MALT lymphoma, and others more controversial, for example, idiopathic thrombocytopenic purpura. More recently, intriguing suggestions that *H. pylori* has protective effects in GERD and autoimmune diseases, such as asthma, have gained momentum. Therefore, we consider the basis for these suggestions and discuss the potential impact for future therapeutic rationales.

1. Introduction

After a long history of discoveries on the pathology and bacterial colonization of the gastric mucosa starting in the beginning of the last century [1], the gastroenterologist Barry Marshall and the pathologist Robin Warren, in the 1980's, fulfilled Koch's postulates for the association between gastritis and the human gastric pathogen *Helicobacter pylori* [1–3]. This decisive demonstration substantially changed our views of the microbiology and pathology of the human stomach and resulted in Marshall and Warren receiving the 2005 Nobel Prize in Physiology and Medicine.

Marshall and Warren's discovery founded the concept that infection with *H. pylori*, and not (if at all, very indirectly) stress, can lead to a variety of upper gastrointestinal disorders (Figure 1) such as gastric inflammation (gastritis), peptic ulcer disease (10%–20%), distal gastric adenocarcinoma (1%–2%), and gastric mucosal-associated lymphoid tissue (MALT) lymphoma (<1%) [4–7]. These insights not only dramatically improved the management and therapy

of gastric diseases but also provided an invaluable key for deeper insights into the pathogenesis of chronic infections. Moreover, during the past 20 years of research, the initially tentative association between persistent *H. pylori* infection and the development of gastric cancer has been well established [8–10], prompting the International Agency for Research on Cancer to classify *H. pylori* as a type I carcinogen [11].

While the prevalence of *H. pylori* is decreasing in the Western world, it still constitutes a significant medical burden in less industrialized countries, with constant higher infection rates and a more widespread distribution. The challenge now is to fill in the remaining gaps in our knowledge such as transmission routes as well as genetic preconditions and, as antibiotic resistance increases, develop preventative strategies, including improved hygiene conditions and vaccination.

Here, we review the role of *H. pylori* in severe human gastric disease such as peptic disease and gastric cancer, with an emphasis on clinical aspects and molecular pathogenesis.

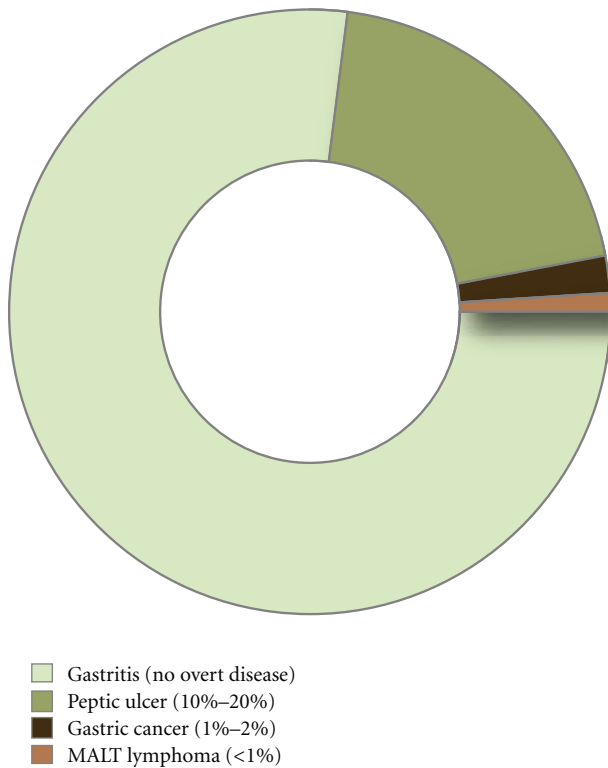


FIGURE 1: Frequency of *H. pylori*-associated human disease. All *H. pylori*-infected individuals develop gastric inflammation (gastritis). 10–20% develop peptic ulcers, whereas gastric cancer occurs in 1–2% of cases. A minority develop MALT lymphoma (<1%).

2. Evolution and Epidemiology

The relationship between *H. pylori* and the human race began around 100,000 years ago. Phylogenetic simulations predict that the bacterium spread from East Africa over the same time scale as anatomically modern humans. The close association is underlined by observations showing that key patterns of bacterial genetic diversity are mirrored in the migration and ethnic origins of the human host [12]. By genotyping different strains collected from all over the world, the migration of humans into North America and the Pacific Area has been tracked [13, 14]. Because a variety of gastric *Helicobacter* species can also be found in mammals besides humans [15], it is speculated that *Helicobacter* species are, in general, ancestral in mammals, and we may have already been infected by ancestors of the present *H. pylori* strains prior to our evolution towards modern mankind [16].

3. Prevalence

It is estimated that half of the world's population is infected with *H. pylori*; however, infection prevalence shows large geographical variations (Figure 2). For example, in several emerging nations and developing countries including India, Saudi Arabia, and Vietnam, more than 80% of the human population is infected. Infections in these regions are characterized by rapid acquisition even at a young age [17–19]. By contrast, the prevalence of *H. pylori* in industrialized

countries is generally less than 40% and considerably lower in children than in adults [20]. *H. pylori* acquisition most frequently appears during early childhood [21], indicating that the decreased prevalence with age is largely due to a birth cohort effect rather than to new infections. The prevalence of *H. pylori* is inversely correlated with socioeconomic status, in particular in relation to family income levels, hygiene, and housing conditions [22]. This may be the reason why the prevalence of *H. pylori* in the industrialized world is significantly declining, whereas infection rates in developing countries remain relatively constant. Moreover, the widespread use of antibiotics may have accelerated the progressive decrease of *H. pylori* infection during childhood in developed countries. The elimination of *H. pylori* from the population by improved hygiene, housing conditions, and antibiotic treatment also strongly correlates with a decrease in gastric cancer worldwide [23].

4. Transmission

Although the association between *H. pylori* and severe human disease has been established, the exact mechanisms of *H. pylori* acquisition are still unknown (Figure 3). Gastro-oral (e.g., exposure to vomit) and fecal-oral routes from human to human are posited as the primary means of transmission [21]. Indeed, *H. pylori* isolates from children and their mothers often have the same genotype [24, 25], supporting the notion that infection primarily occurs during childhood via close contact to family members (e.g., via pre-mastication of food by parents). Other transmission routes, including exposure to contaminated food or water [26–28] or via domestic animals such as cats and sheep [29, 30] are also thought to play a role. To date, no conclusive evidence of predominant transmission by any of these routes has been established.

5. Colonization and Persistence

The majority of colonizing *H. pylori* reside within the gastric mucus and do not directly interact with host cells. Although *H. pylori* was long considered to be an extracellular bacterium, recent studies have provided evidence that *H. pylori* occasionally enters epithelial cells via a zipper-like mechanism [31–35]. Despite causing numerous gastric environmental changes and eliciting a host immune response, *H. pylori* can persistently colonize the human stomach for long periods [36]. This is in stark contrast to most other commonly ingested microbes, which cannot successfully colonize the stomach due to its manifold microbicidal properties.

6. Mechanisms to Escape Low pH

One of the most efficient biological barriers against bacterial infection is the acidic pH (<2) of the gastric lumen. However, the mucus layer overlying gastric epithelial cells exhibits a pH gradient, ranging from about pH 2 at the luminal surface to between 5 and 6 at the epithelial surface [37].

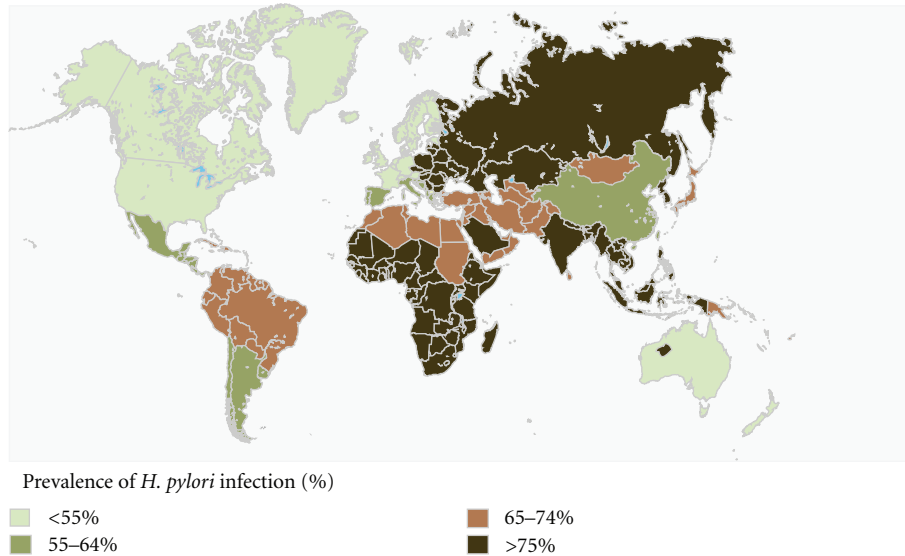


FIGURE 2: Worldwide prevalence of *H. pylori* infection. Infection rates in percent. *H. pylori* infection is highly prevalent in Africa, Asia, and South America.

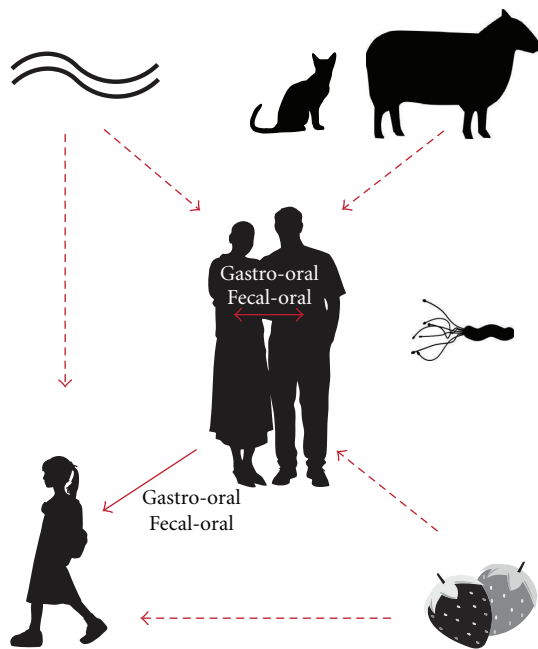


FIGURE 3: *H. pylori* transmission routes. Exact transmission routes are still not known. Person-to-person transmission by either the gastro-oral or fecal-oral route is most likely. Several studies indicate transmission via water, pets and food, but the majority of the data support the hypothesis that infection primarily occurs during childhood via close contact to family members especially from mother to child.

To avoid the bactericidal activity of acid, *H. pylori* produces substantial amounts of cytosolic and cell surface-associated urease. Urease is highly conserved in different *H. pylori* strains and transiently buffers the acidic environment by converting urea into ammonia and carbon dioxide [18, 38]. *H. pylori* has evolved several other strategies to minimize

exposure to the low pH in the gastric lumen (see Figure 4). The bacteria remain within the mucus in close proximity to the epithelial surface where the pH is nearly neutral. The spiral cell shape of *Helicobacter*, which is based on specific cross-linkages of the peptidoglycan layer, supports a screw-like movement which facilitates motility within the viscous mucus layer and enhances colonization efficiency [39, 40]. To rapidly reach and remain in the mucus layer, *H. pylori* uses polar flagella [41, 42]. Interestingly, null mutants defective in the production of flagella are unable to colonize gnotobiotic piglets but grow normally *in vitro* [43]. Consistent with the importance of functional flagella, control of directed movement by chemotactic responses is also essential for successful colonization [44]. Intriguingly, during infection of Mongolian gerbils *H. pylori* orient themselves via the pH gradient in the mucus, remaining mainly within 25 μm of the epithelial surface and thereby avoiding acidic distal regions [45]. A specific chemoreceptor, encoded by the *tlpB* gene, seems to be involved as mutants can swim but do not avoid acidic regions [46].

7. Adhesion to Epithelial Cells

Only ~20% of *H. pylori* present in the gastric mucosa are adhered to the surface of epithelial cells with a majority exhibiting a tropism for intercellular junctions and, occasionally, for deeper intercellular spaces [47, 48]. Adherence is mediated by a subset of *H. pylori*-encoded autotransporter proteins (e.g., BabA, SabA, AlpA, AlpB, HopZ, and OipA) exposed on the bacterial cell surface. However, no individual molecule has been shown to be essential, indicating redundancy of adhesive mechanisms [49–52]. Additionally, expression of individual adhesins differs between strains and is variable within a single strain over time, leading to dynamic adaptation capacities via on/off switching of gene expression, gene inactivation, or recombination [53–55].

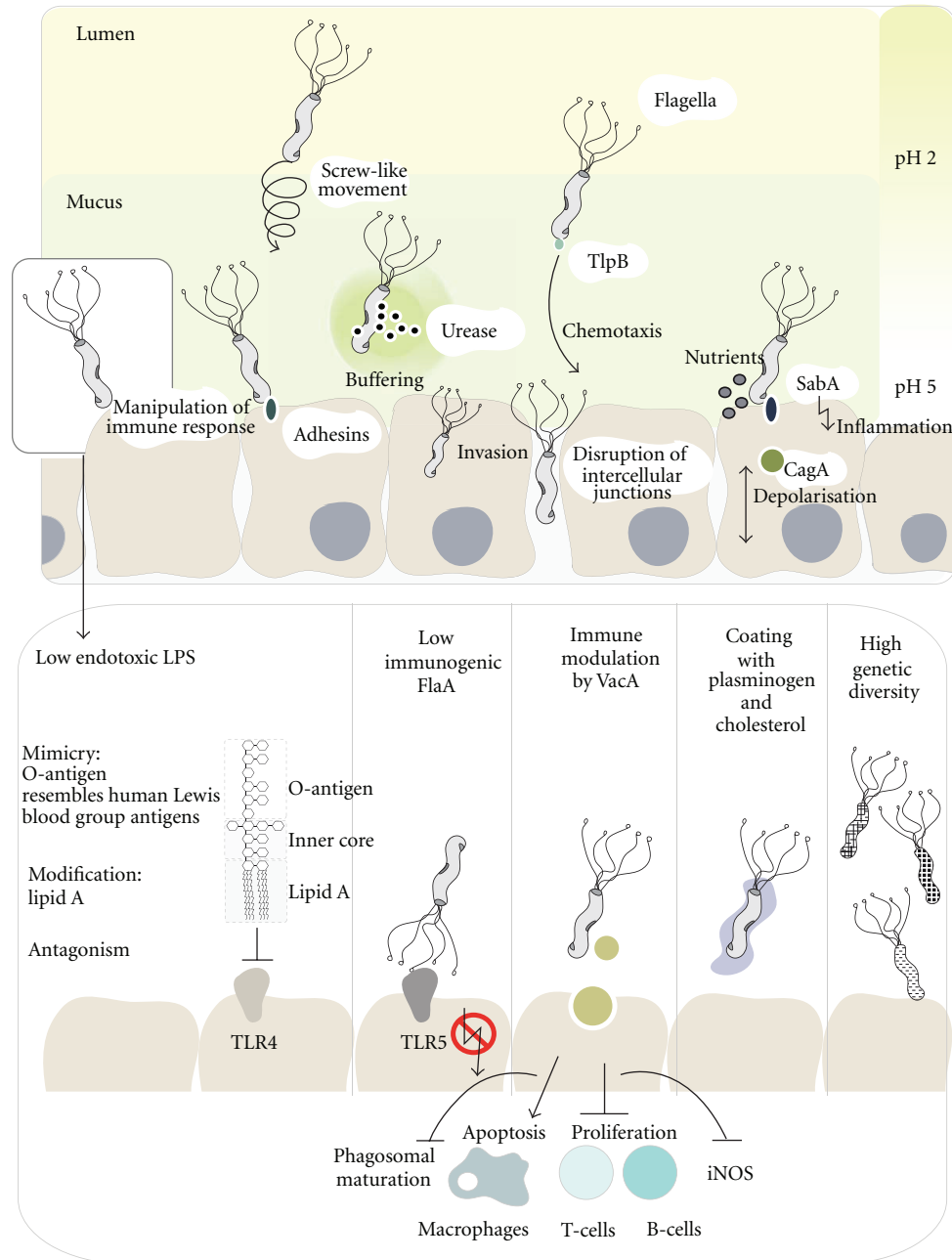


FIGURE 4: Molecular mechanisms of bacterial colonization and persistence. *H. pylori* has evolved numerous strategies to facilitate colonization and persistence in the hostile environment of the human stomach. Multiple polar flagella allow fast motility. Using the flagella, the bacteria orientate themselves towards the mucosa in close proximity to the epithelial cell layer where the pH is near neutral. The chemotaxis receptor TlpB is involved. Movement through the viscous mucosal layer is facilitated by a helical cell shape, which enables a screw-like movement. The cytosolic and membrane-associated bacterial enzyme urease buffers the environment by catalyzing the hydrolysis of urea, forming ammonia and carbon dioxide. Multiple bacterial adhesins mediate bacterial attachment to the epithelial cells, occasionally followed by invasion into the cells or traversing between intercellular junctions. Bacterial factors such as the adhesin SabA or the translocated effector protein CagA support the release of important nutrients to the apical side of the epithelial cells, either via the induction of inflammation or by the depolarization of the host cell. Persistent colonization is also enabled by a complex manipulation of the immune system. Specific modifications of LPS molecules enable molecular mimicry and structural alterations of the lipid A component which lead to low endotoxic activity. Additionally, *H. pylori* LPS can antagonize the pattern recognition receptor TLR4. The main flagella component FlaA also exhibits only weak TLR5-mediated immunogenic host cell responses, and the vacuolating toxin VacA can strongly interfere with immune cells by inhibiting T- and B-cell proliferation or phagosomal maturation of macrophages. VacA can also induce apoptosis of macrophages. The coating of the bacterial membrane with host cell molecules such as plasminogen and cholesterol protects *H. pylori* from recognition by the host, and the high genetic bacterial diversity allows fast adaptation to changes in the environment. TLR, Toll-like receptor; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthase.

8. Manipulation of the Immune System

H. pylori can persist for long time periods within the host without being extinguished by the immune system or by the frequent gastric environmental changes. Interactions of *H. pylori* with host epithelial surfaces are thought to elicit effective escape mechanisms, often causing cellular damage and inflammation. For example, intimate adherence via SabA has been shown to enhance inflammatory responses, which are assumed to facilitate access to essential nutrients released from damaged host cells [56]. Although direct evidence is lacking that inflammation is beneficial for *H. pylori*, the hypothesis still remains intriguing given that infection always elicits inflammation, regardless of symptoms. In addition, the delivery of cytotoxin associated antigen A (CagA), a key virulence factor, into the host cell via the type four secretion system (T4SS), depolarizes the epithelial cell to exploit the apical cell surface for use as a replicative niche and to obtain nutrients which are normally delivered to the basolateral side [57].

In general terms, immunity does not seem to exert a decisive influence on the establishment of an infection, as immune-compromised patients do not exhibit higher colonization rates [58]. Besides, adherence and the concomitant delivery of toxins, *H. pylori* possess a range of mechanisms to attenuate and manipulate the immune response, for example, the bacteria preferentially induce a T-helper cell 1 [Th1] type-based response, classified as typical cell mediated immunity, essential for the fight against intracellular pathogens [59]. *H. pylori* also expresses lipopolysaccharide (LPS) with a very low endotoxic and immunobiological activity compared to LPS of other Gram-negative bacteria [60] that can antagonize signaling mediated by the innate immune receptor, toll-like receptor 4 (TLR4) [61]. This antagonistic feature is based on specific modifications of the lipid A component [62] and strain-dependent expression of LPS O-antigens that are structurally related to Lewis blood group antigens found on human cells [63]. This molecular mimicry is not only involved in autoimmune responses but could also allow *H. pylori* LPS to evade recognition by the innate immune system [64]. The incorporation of cholesterol and its subsequent glycosylation in the *H. pylori* membrane [65] as well as the coating of the bacterium with host molecules such as plasminogen [66] might represent additional mechanisms of antigenic camouflage. Moreover, *H. pylori* flagella, structures which are normally recognized via the innate immune receptor TLR5, evoke only a very weak immune response due to a modified N-terminal TLR5 recognition site [67–69]. Other mechanisms by which *H. pylori* triggers the host immune system are based on bacterial factors that directly target host immune cells; for example, the vacuolating cytotoxin (VacA), which is encoded in the genome of all *H. pylori* strains, inhibits the nuclear translocation of the transcription factor nuclear factor of activated T-cells (NFAT), thereby blocking T-cell proliferation of CD4⁺ cells [70]. VacA also inhibits the proliferation of B-cells and CD8⁺ cells [71] and is able to disrupt the normal function of macrophages by either inducing apoptosis [72] or interrupting phagosomal maturation [73].

Bacterial arginase and γ -glutamyl transferase show similar functions in altering the normal function of T-cells [74, 75], while arginase and VacA additionally downregulate the expression of inducible nitric oxide synthase [76, 77].

9. Genetic Diversity and Variation

H. pylori exhibits a remarkable allelic diversity and genetic variability, typically involving endogenous (point) mutations and recombination, which result in every infected person carrying a distinct strain [78, 79]; however, differences between relatives are minimal [80]. Allelic diversity is promoted by a significantly higher mutation rate than found in many other bacteria [81], which may also explain the rapid development of high-level resistance to commonly used antibiotics such as clarithromycin. High mutation rates are most likely due to the lack of a complete DNA mismatch repair system (*mutS1/MutL/mutH*) and several enzymes involved in base excision repair [82, 83]. A large repertoire of hyper-mutable genes frequently undergoes length changes as a consequence of slipped-strand mispairing-mediated mutagenesis, leading to numerous subpopulations within any large population [84]. Each of these subpopulations carry a specific combination of active and inactive phase-variable genes; for instance, LPS biosynthesis and outer membrane encoding genes, which allow *H. pylori* to rapidly adapt to environmental changes [85, 86]. High natural competence for DNA uptake in combination with one of the highest intergenomic recombination rates found amongst pathogenic bacteria [87] also contributes to the high genomic variability between *H. pylori* isolates. Moreover, recent work has demonstrated that genetic exchange induced by damage of bacterial DNA contributes to persistence of *H. pylori* in its host [88]. Interestingly, a recent microarray analysis identified a large number of genes not previously associated with infection as essential for colonization of mice [89]. The majority of gene products were hypothetical, indicating that many other unknown bacterial factors may be required for colonization.

10. Gastric Cancer and Ulcers: Mutually Exclusive Pathologies?

10.1. Gastric Cancer. Although many *H. pylori*-associated diseases including peptic ulcer, gastric cancer, and MALT lymphoma only develop decades after infection (Figure 5), their medical burden is tremendous. Gastric cancer is one of the most common forms of cancer, with approximately 700,000 to 900,000 new cases diagnosed every year, and the second leading cause of cancer-related deaths worldwide [90]. Survival rates are very low, ranging from 15% if diagnosed during later stages of the disease to 65% if diagnosed early. Incidence rates vary widely geographically, and, in general, more males than females are affected [50% lower incidence]. Although high-risk areas in Japan, China, Eastern Europe, and certain Latin America countries still remain [91], incidence rates worldwide have been declining for several decades [92].

Gastric adenocarcinomas are mainly divided into two histologically distinct forms, diffuse-type gastric adenocarcinoma, and intestinal-type adenocarcinoma (Lauren classification) each exhibiting different epidemiological and pathophysiological features [93]. Diffuse-type gastric adenocarcinoma is found predominantly in younger people, with no gender bias. It consists of individually infiltrating neoplastic cells that do not form glandular structures and are not associated with intestinal metaplasia [94]. The more prevalent form of gastric adenocarcinoma is called intestinal-type adenocarcinoma, which usually occurs in elderly people, predominates in men, and progresses through a well-defined chain of histological events, typically starting with a transition from normal mucosa to chronic gastritis, followed by atrophic gastritis and intestinal metaplasia which finally ends in dysplasia and adenocarcinoma [95]. *H. pylori* significantly increases the risk of developing both cancer types. We focus here mainly on the association between *H. pylori* and intestinal-type adenocarcinoma as the mechanisms of disease progression are better characterized as compared to diffuse-type gastric adenocarcinoma. Gastric cancers are also classified by their localization within the stomach: the most important distinction being between cardia (the proximal part of the stomach) and noncardia. *H. pylori* is the strongest risk factor for the development of non-cardia (distal) gastric cancer.

10.2. Peptic Ulcer Disease. Due to the high morbidity and mortality rates associated with peptic ulcer disease, which comprises both gastric and duodenal ulcers, this disease is regarded as a major medical threat. In most Western countries, morbidity from duodenal ulcer is more common than from gastric ulcer, even though the mortality rate is higher for gastric ulcer. In Japan, morbidity and mortality rates are generally higher for gastric ulcer than for duodenal ulcer [97]. Both ulcer types are defined as mucosal defects with a diameter of at least 0.5 cm penetrating through the muscularis mucosa. Whereas gastric ulcers mainly occur along the lesser curvature of the stomach, at the transition from corpus to antrum [98], duodenal ulcers can be normally detected in the duodenal bulb, or even with the pylorus. As with gastric cancer, recent epidemiological studies show a sharp decline in the incidence of ulcer disease [91], a direct consequence of the disease association with *H. pylori* and the now-possible effective antibiotic medication. Although the identification of *H. pylori* and its association with ulcer development completely changed the prevailing concept of “no acid, no ulcer”, nonsteroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin are also an increasingly important cause of ulcers, even in *H. pylori*-negative patients [99].

11. What Makes the Difference between Cancer and Ulcer?

Although both gastric cancer and peptic ulcer disease are associated with *H. pylori* infection, mechanistically they appear to have entirely different origins and outcomes

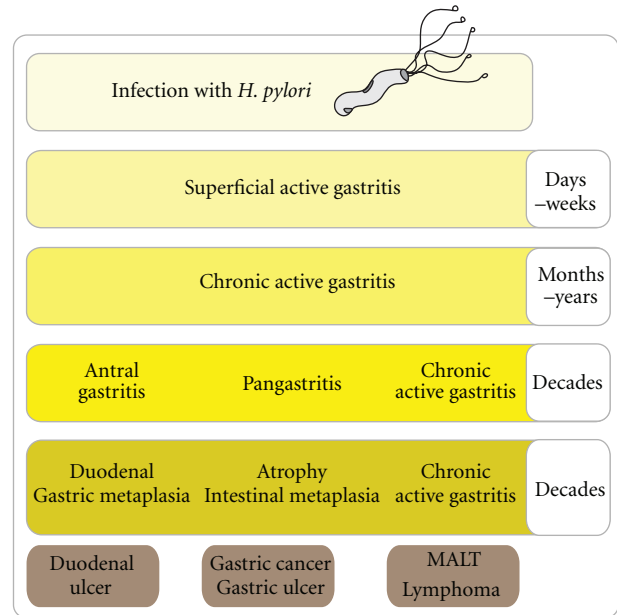


FIGURE 5: Time line of disease progression in *H. pylori*-infected persons. All infected individuals develop a superficial gastritis within the first weeks of infection, followed by a chronic active gastritis which develops after months or years. After decades, patients can develop antral gastritis or pangastritis, depending on the localization of the infection. The antral inflammation can lead to gastric metaplasia, which supports the growth of duodenal ulcer. The latter can lead to atrophy and intestinal metaplasia, two prerequisites for the development of gastric cancer or gastric ulcer. In contrast, constant chronic active gastritis can lead to the growth of MALT lymphomas. Adapted from Telford et al. [96].

(Figure 6). Gastric adenocarcinomas are malignant tumors which arise from uncontrolled proliferation of the epithelial cell layer and are accompanied by hypochlorhydria (low-acid secretion). The tumors mainly occur in the gastric antrum, body, or, to a lesser extent, in the fundus and originate from the inflammation of the entire stomach (pangastritis). By contrast, a peptic ulcer is a sore on the lining of the stomach (gastric ulcer) or duodenum (duodenal ulcer; DU) and originates from a disruption of normal wound-healing processes of the epithelial layer. Whereas acid secretion in duodenal ulcer is increased (hyperchlorhydria), gastric ulcers develop in low acid concentrations, as also observed with gastric cancer. This discrepancy is also due to the differences in the origin of prior inflammation, that is, predominantly in the antrum in cases of duodenal ulcer, but involving the entire stomach in cases of gastric ulcer [5]. While gastric cancer and duodenal ulcer can be easily discriminated from one other, gastric ulcer more closely resembles gastric cancer in localization and outcome. This relationship is also reflected in the observation that gastric cancer often occurs in patients with a history of gastric ulcers but hardly ever in patients with recent duodenal ulcers [100]. Moreover, gastric ulcer and gastric cancer patients are characterized by reduced acid secretion, corpus-predominant pangastritis, and an accelerated progression towards atrophic gastritis and intestinal metaplasia. The differences in gastric cancer and

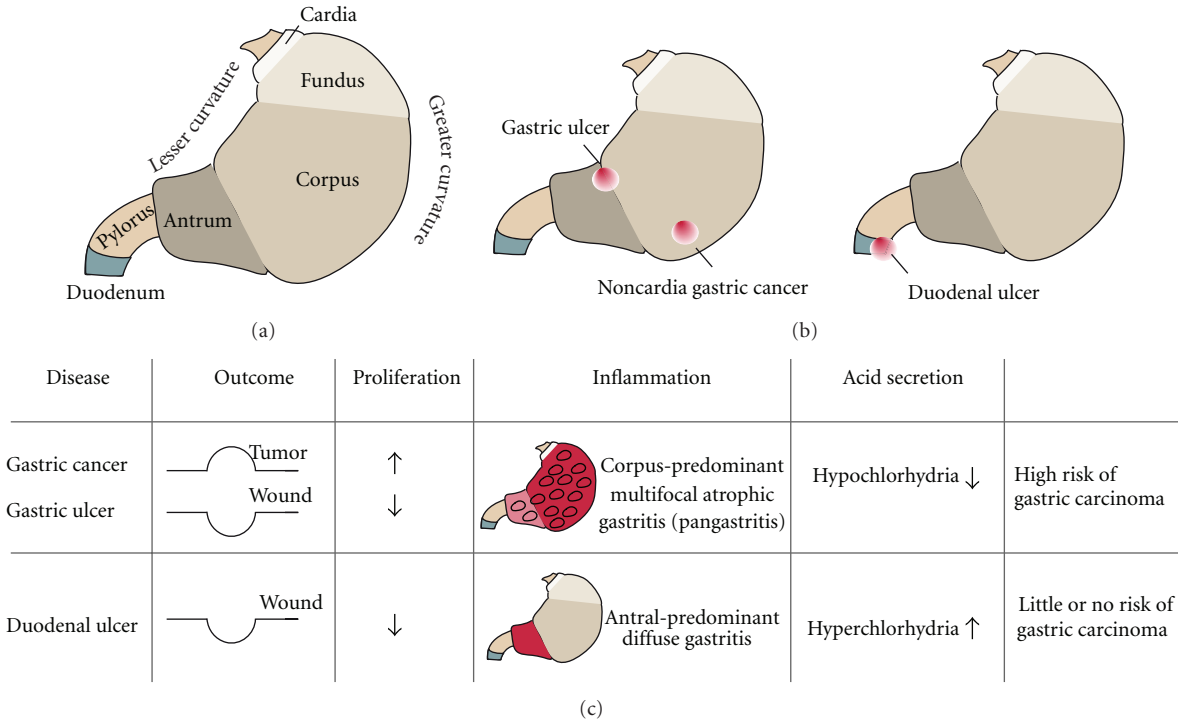


FIGURE 6: Characteristics of *H. pylori*-mediated human disease. (a) Anatomical structure of the human stomach. (b) Localization of peptic ulcer (gastric and duodenal ulcer) and noncardia gastric cancer in the human stomach. (c) Differences in outcome, proliferation, inflammation, acid secretion, and risks for the development of gastric carcinoma.

duodenal ulcer are also mirrored in their worldwide prevalence, but not cooccurrence (Figure 7). Duodenal ulcer incidence is mainly concentrated in South Asia (e.g., India) and West Africa, whereas gastric cancer is mainly prevalent in Middle Asia and Middle Africa. Interestingly, some African (and Indian) populations have a high prevalence of *H. pylori* but low incidence of *H. pylori*-associated diseases, termed the “African enigma” [101]. One possible hypothesis posited for this anticorrelation is that enteric helminth infection in developing countries can attenuate *H. pylori*-induced atrophy and premalignant lesion by modulating the Th1-driven immune response to bacterial infection [102, 103].

12. *H. pylori* Virulence Determinants

12.1. Strain Variations Trigger the Pathogenic Outcome of *H. pylori* Infections. The phenomenon that most *H. pylori*-infected patients develop no complications other than chronic active gastritis led to the notion that some strains may be more virulent than others. In fact, early investigations indicated that the severity of pathogenic outcome was correlated with the strain’s ability to induce strong morphological changes, vacuolization and degeneration of *in vitro* cultured cells [104]. Because *H. pylori* can colonize the entire gastric epithelium, clinical outcomes are mainly dependent on the pattern of chronic inflammation induced. Therefore, bacterial factors involved in *H. pylori*-induced inflammatory responses constitute risk factors for both gastric cancer and peptic ulcer. Despite the

wide genetic diversity of *H. pylori* hampering the search for bacterial factors involved in pathogenesis, several genomic loci encoding virulence factors, such as the *cag* pathogenicity island (*cagPAI*), the toxin *VacA*, and the *Bab2* adhesin, have been strongly associated with an increased risk of developing gastric cancer and peptic ulcer disease [105, 106]. Like disease prevalence, the distribution of these genomic loci varies geographically. While the *VacA*-encoding gene can be found in all strains, albeit with various allelic combinations, only 60–70% of all Western strains carry *cagPAI*. In contrast, *cagPAI* is present in 95–100% of all Asian strains. The *bab2* gene is present in around 85% of all strains.

12.2. The *cag* Island and the Virulence Factor *CagA*. One of the most important strain-specific determinants influencing *H. pylori*-mediated pathogenesis is *cagPAI*. This horizontally acquired genomic locus of approximately 40 kb contains 31 genes [83, 107] that encode a T4SS. It was first detected while searching for the genomic localization of the bacterial protein *CagA*, a marker for *H. pylori*-associated diseases [108]. The *cagA* gene, located at the 3’ end of *cagPAI*, encodes the only known bacterial effector protein translocated by the T4SS into host cells. The T4SS forms a membrane protrusion, often inaccurately referred to as a needle-like structure [109, 110]: it is not comparable to the static form of a bacterial type 3 secretion system [T3SS], which truly resembles an injection syringe [111]. *CagA* is often detected on the surface of the T4SS; it becomes translocated into epithelial cells and to a lesser extent into hematopoietic cells

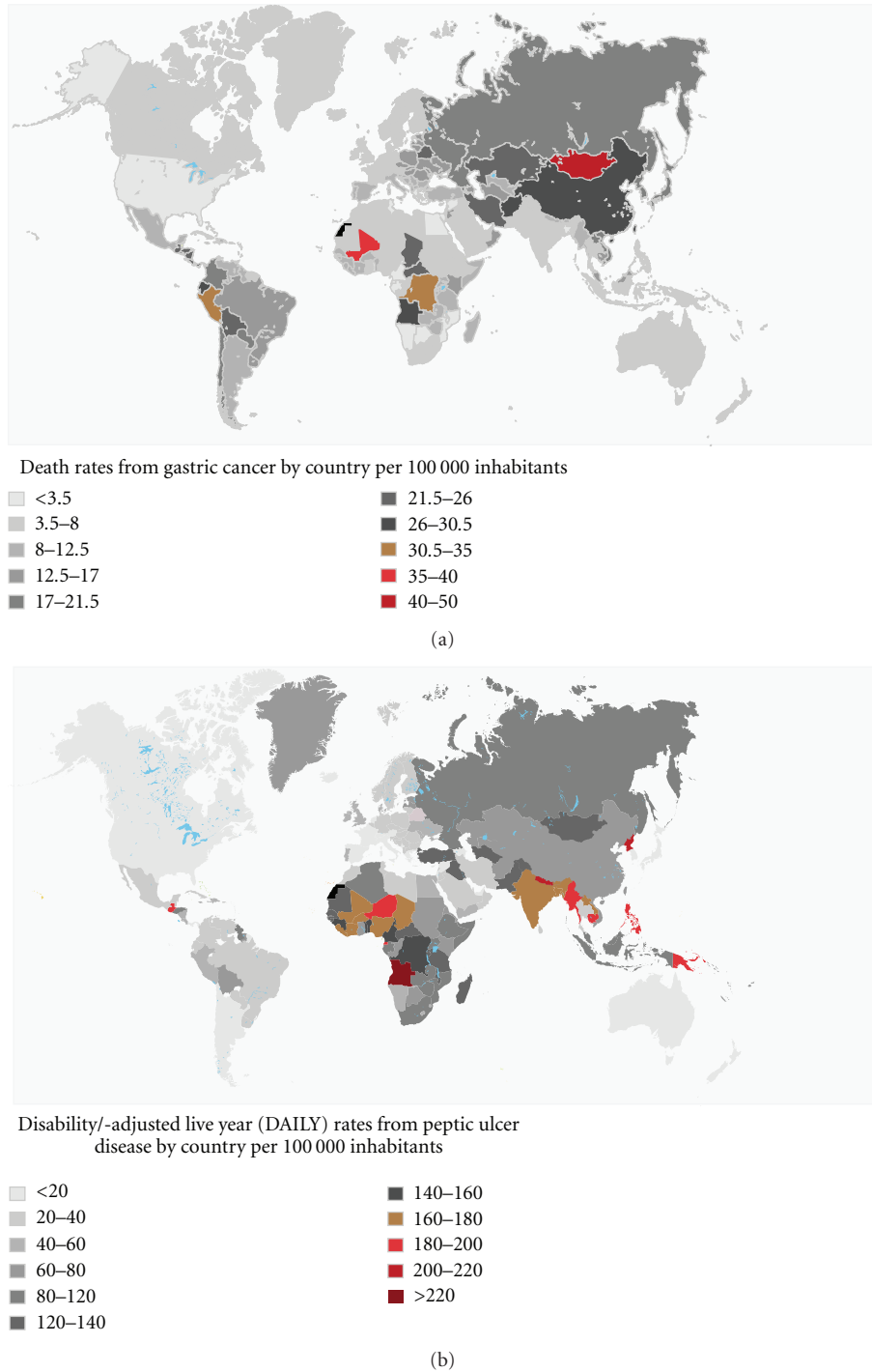


FIGURE 7: Worldwide gastric cancer death rates and peptic ulcer disability rates. *H. pylori* infection prevalence mirrors the outcome of *H. pylori*-associated disease. The highest gastric cancer death rates are concentrated in Middle Asia and Middle Africa, whereas the peptic ulcer causes disabilities mainly in West Africa and South Asia. Data derived from Death and DALY estimates for 2004 by cause for WHO Member States (Persons, all ages) (2009-11-12).

[112, 113]. Intriguingly, CagA has also been detected on the surface of outer membrane vesicles (OMVs) secreted by *H. pylori* [114]. The interdependence of T4SS function, OMV formation, and CagA translocation likely remains an interesting area of future investigation.

Surface-exposed integrin molecules act as host cell receptors which permit the successful delivery of CagA and, thus, play a key role in T4SS functionality. In this context, CagL, a T4SS component, was proposed to have an important function by mediating binding between $\beta 1\alpha 5$ integrins and

its RGD-domain [115]. Although contradictory data have been published [116], recent observations could support the CagL-integrin interaction concept by showing that CagL activates the integrin-binding molecule ADAM17 via mediating its dissociation from β -integrin [117]. Accordingly, ADAM17 inhibits the expression of the gastric H^+ , K^+ -ATPase α subunit via the transcription factor nuclear factor κB (NF- κB). Since the H^+ , K^+ -ATPase α subunit is involved in acid secretion, these data demonstrate a T4SS-dependent molecular mechanism for hypochlorhydria and indicate that the influence of *cagPAI* on the development of gastric cancer and gastric ulcer is more pronounced than on the induction of duodenal ulcer (Figure 8). Additional *cag*-encoded proteins (CagA, CagI and CagY) have been shown to bind $\beta 1$ -integrin in order to induce conformational changes of integrin heterodimers which enable CagA translocation [116]. Since integrins are not located at the apical membrane but are expressed at the basolateral side, *H. pylori* would have to reach the basolateral surface to efficiently activate the T4SS. Moreover, in addition to the normal expected apical niche, viable bacteria have been observed within paracellular spaces and the gastric lamina propria [47]. Accordingly, *H. pylori* induces the shedding of E-cadherin to disrupt adherence junction complexes in a CagA-independent manner [118], enabling bacteria to enter the basolateral side of formerly closed epithelial cell layers (Figure 8).

The status of CagA as a marker of pathogenic disease resulted from the observation that patients with elevated antibody titers against CagA showed higher incidences of both peptic ulcers [119] and gastric adenocarcinoma [120, 121]. The association of CagA with gastric cancer was cemented by subsequent work showing that the expression of CagA in transgenic mice leads to gastric epithelial cell proliferation and the development of gastric adenocarcinoma [122]. Data showing CagA-dependent attenuation of apoptosis in Mongolian gerbils [123] and that CagA-induced blockage of endocytosis mediated the downregulation of the proliferation controlling epidermal growth factor receptor (EGFR) [124] further support the hypothesis that CagA has oncogenic features. CagA can also provoke proinflammatory responses [125] and activate the signal transducer and activator of transcription 3 (STAT3) pathway, thought to play a role in gastric cancerogenesis (Figure 8) [126]. Nevertheless, strains lacking *cagPAI*, which consequently do not express CagA, have also been found in patients with peptic ulcers or gastric cancer, albeit at lower frequencies. The mechanisms by which CagA mediates gastric human disease are still not fully understood. Future work is required to unravel the complex but extremely efficient manipulation of important host cell signaling cascades facilitated by this multifunctional protein.

Recent investigations have shown that surface-exposed CagA interacts with externalized phosphatidylserine to initiate its entry into host cells [127]. Following its injection into epithelial cells, CagA undergoes tyrosine phosphorylation [128, 129], mediated by the nonreceptor tyrosine kinases Src [130] and Abl [131], two well-known oncoproteins (Figure 8). Phosphorylation is tightly controlled by *H. pylori* in a time-dependent manner; Src is only activated during

initial stages of infection (0.5–2 h) [132], whereas Abl is strongly activated at late infection time points (2–8 h) [133]. Phosphorylation takes place at specific C-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) sequence motifs of CagA which can vary in number in a strain-dependent manner [134, 135]. Four EPIYA-sites—A, B, C, D—have been identified. The EPIYA-A and EPIYA-B motifs are distributed worldwide, whereas the EPIYA-C motif is more prominent in strains from Western countries (e.g., Europe, Australia, North America) and some Asian countries (Malaysia and India). EPIYA-D sequence motifs are mainly present in East Asian CagA (Japan, China, and Korea) and are linked to the development of gastric cancer [136].

It is not only the sequence motif but also the number and variation of phosphorylation sites that determine the pathogenic potential of CagA. Elevated phosphorylation, caused by an increase in number of EPIYA sites, leads to enhanced binding of another oncoprotein, the tyrosine phosphatase SHP-2. After binding to CagA, SHP-2 is functionally deregulated [137]. CagA recruits and activates SHP-2 in a phosphorylation-dependent manner and induces a Ras-independent response which leads to dramatic morphological changes of the host cell, reflected by a strong actin polymerization and cellular elongation (Figure 9), termed the “hummingbird” phenotype [138]. Expression of EPIYA-A and EPIYA-B sites correlate with the ability of CagA to bind the tyrosine kinase Csk, which in turn inhibits Src-dependent CagA-phosphorylation, thereby, attenuating the induction of cellular elongation by the CagA-SHP-2 complex [139]. These contradictory functions of different EPIYA sites highlight the complex and multifunctional role of CagA in *H. pylori*-mediated disease. That CagA interacts with a surprisingly high number of host cell signaling factors further complicates the elucidation of CagA function. Interactions can be phosphorylation dependent or independent, and interaction partners range from kinases to adaptor molecules [140]. In addition to CagA-independent interactions reported by Weydig and colleagues [118], a phosphorylation-independent interaction between Zonula occludens-1 (ZO-1), a junctional adhesion molecule, and CagA was shown to contribute to an alternative mechanism of epithelial layer disruption [141]. The CagA-ZO-1 association was closely linked to an ectopic assembly of tight junction components at the site of bacterial attachment which altered the composition and function of the apical junctional complex (see Figure 8).

Binding between CagA and the PARI/MARK kinase complex, which has an essential role in the maintenance of epithelial cell polarity (Figure 8), has also been shown to be phosphorylation independent [142]. Association with CagA inhibits PARI kinase activity leading to the dissociation of PARI from the membrane causing junctional and polarity defects. These effects are dependent on conserved amino acid motifs at the C-terminus of CagA, termed the CagA multimerization (CM) domain [143]. The binding of PARI by the CM domain mediates the dimerization of CagA, thereby, strengthening the bond between CagA and SHP-2. The functional relevance of this association has been further corroborated by cocrystallography analysis of CagA, showing

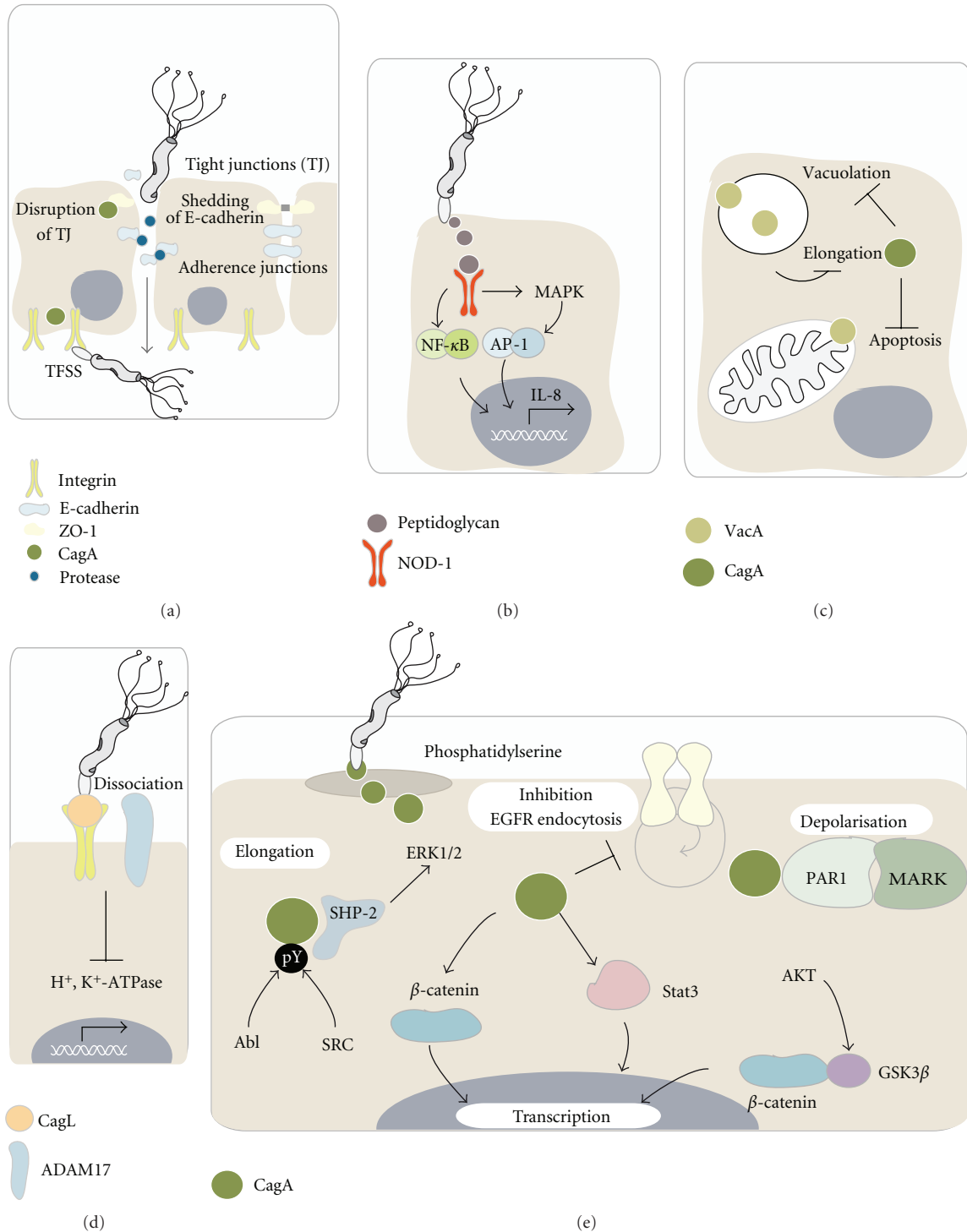


FIGURE 8: Molecular pathogenesis. *H. pylori* has evolved different mechanisms to destroy intercellular junctions. (a) The secretion of a bacterial protease leads to the shedding of E-cadherin, an important component of adherence junctions. The toxin CagA, which is translocated in the epithelial cell by the bacterial T4SS, interacts with ZO-1, thereby, destroying the formation of tight junctions. By crossing the intercellular space, *H. pylori* reaches basolateral integrins which function as T4SS receptors. (b) The T4SS also delivers peptidoglycan into the host cell. Peptidoglycan is recognized by NOD-1 which activates the transcription of proinflammatory genes such as *IL8* via the transcription factors NF- κ B and AP-1. AP-1 is activated via MAP kinases (MAPK). (c) VacA induces vacuolization as well as mitochondria-mediated apoptosis in epithelial cells, whereas CagA induces drastic morphological changes such as cellular elongation. CagA and VacA counteract each other. (d) The T4SS component CagL mediates the interaction between the T4SS and integrins, thereby leading to the dissociation of the metalloproteinase ADAM17, a process which inhibits the expression of the H⁺, K⁺-ATPase. (e) CagA is translocated via phosphatidylserine-rich domains into the host cell where it is phosphorylated via Src kinases and Abl. Phosphorylation leads to an interaction with the tyrosine phosphatase SHP-2 which induces a constant activation of ERK1/2. These events cause cellular elongation. Furthermore, CagA can inhibit EGFR endocytosis thereby blocking receptor degradation. CagA also activates β -catenin and STAT3 which leads to transcription of oncogenes. β -catenin activation can also be CagA independent, induced by an AKT-mediated phosphorylation of the β -catenin inhibitor GSK3 β . Interaction between CagA and the PAR1/MARK complex can lead to a depolarization of the host cell.

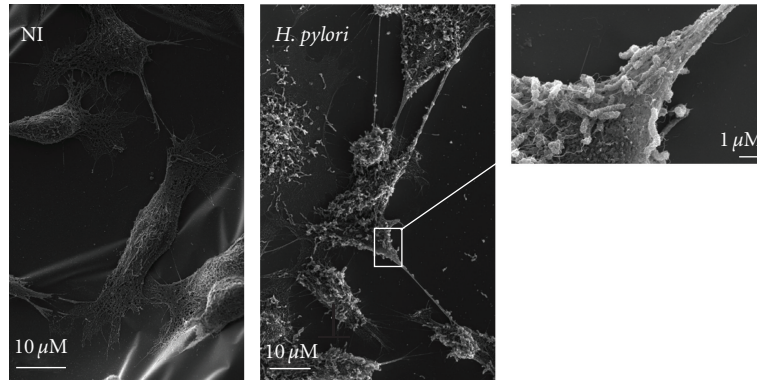


FIGURE 9: *H. pylori*-infected human gastric epithelial cells. Scanning electron microscopy pictures from cytoskeletal preparations of noninfected (NI) and infected (*H. pylori*) human gastric epithelial adenocarcinoma cells (AGS). Infected cells show dramatic morphological changes of the cytoskeletal network.

that the C-terminal CM domain blocks PAR1 function by occupying the substrate-binding site of PAR1 [144]. Thus, the CagA-PAR1 association not only causes polarity defects but also promotes the morphogenic response induced via the interaction of CagA and SHP-2.

Another host cell factor potentially influencing cancerogenic responses in conjunction with CagA is the ubiquitously expressed protein β -catenin (Figure 8). While membrane bound β -catenin is an important component of adherence junctions, cytoplasmic β -catenin is a downstream signaling molecule of the WNT signal transduction pathway. Upon dissociation of the inhibitory β -catenin-interaction partner GSK3 β , β -catenin is translocated into the nucleus where it induces the transcription of target genes involved in cancerogenesis [145]. After translocation, CagA induces nuclear accumulation and functional activation of β -catenin via the disruption of the membrane-bound E-cadherin/ β -catenin complex [146–148]. *H. pylori* infection was also shown to lead to AKT dependent, but CagA-independent, β -catenin activation through phosphorylation (Figure 8) and subsequent inactivation of the β -catenin inhibitor GSK3 β [149].

Studies of CagA-host cell interactions have provided many fundamental insights into the molecular mechanisms by which *H. pylori* induces potentially cancerogenic host cell responses; however, the mechanisms underlying the association of CagA with ulceration remain elusive. It has neither been postulated nor shown that CagA could promote a delay in wound healing or an inhibition of cell proliferation. By contrast, VacA could inhibit proliferation, whereas undialyzed *H. pylori* blocked both wound healing and cell proliferation, indicating the existence of CagA- and VacA-independent mechanisms leading to the promotion of ulcer development [150]. Future work is undoubtedly required to solve the remaining conundrums.

12.3. The *cag* Island and the Delivery of Peptidoglycan. Besides CagA translocation, the T4SS is also involved in the production of chemokines, such as interleukin-8 (IL-8). This process is mediated by the combinatorial activation of the host cell transcription factors NF- κ B [151] and AP-1, the latter activated by the MAPK pathway [152, 153].

MAPK phosphorylation and activation of AP-1 are dependent on the intracellular pattern recognition factor NOD-1 (nucleotide-binding oligomerization domain-containing protein 1) [154], which is known to recognize universal bacterial components such as peptidoglycan. Indeed, peptidoglycan was suggested to be translocated into the host cell in a T4SS-dependent manner [155], thereby, inducing the activation of NF- κ B via NOD-1 (Figure 8). Delivery is mediated via cholesterol-rich microdomains (lipid rafts) of the host cell plasma membrane [156]. Delivery of peptidoglycan, however, can also occur independently of the TFSS, instead mediated by bacterial OMVs which enter human gastric epithelial cells via lipid rafts [157]. This mechanism provides an explanation for the varying dependency of IL-8 secretion in different epithelial cell lines on the T4SS [113]. The cell-line specificity of TFSS-independent IL-8 secretion could be due to variations in the composition of lipid raft associated receptors, enabling TFSS-dependent peptidoglycan delivery and/or OMV-mediated peptidoglycan translocation.

Both CagA and peptidoglycan seem to be responsible for the induction of a T4SS-dependent proinflammatory response, although the ability of CagA to mediate IL-8 expression is not identical for all CagA-expressing strains [125, 158]. Observations that CagA and NOD-1 accumulate in a novel type of intracellular host cell structure enforce the model that CagA and peptidoglycan can induce immune-inflammatory and proliferative responses in the gastric epithelium with potential pathologic relevance [159]. Recent work, however, shows that the deletion of the bacterial deacetylase HP0310, which is required for normal synthesis of peptidoglycan, results in an increased delivery of CagA into host cells [160], indicating that even in the absence of peptidoglycan *H. pylori* retains the ability to initiate a proinflammatory response.

12.4. Vacuolating Toxin VacA. *H. pylori* VacA is a paradigmatic type-V-secreted bacterial toxin that contributes to the establishment of successful infection and virulence in multiple ways. Similar to CagA, it has been shown to be responsible for epithelial ulceration [161]. VacA was initially identified

through its ability to cause vacuolation in cultured epithelial cells [104]. VacA-induced vacuoles are positive for marker proteins of the late endocytic compartment, including Rab7 [162, 163], LAMP1, and Lgp110 [164]. It is supposed that VacA induces the formation of large vacuoles after its internalization into endosomal structures, where it forms anion-selective channels, subsequently leading to swelling of VacA-containing endosomal compartments [165]. Although vacuolation is readily observed *in vitro*, it does not seem to occur *in vivo* [5].

The VacA amino acid sequence shares no similarities with other prokaryotic or eukaryotic proteins, and although all strains contain *vacA*, their sequences vary remarkably. *vacA* alleles are distinguished by differences in the 5' region (s-region) and midregion (m-region). Strains possessing s1m1 type alleles are associated with an increased risk of peptic ulceration and gastric carcinoma as compared to strains harboring other allelic forms, for example, s2m2 [166, 167]. In countries with high rates of distal cancer, such as Colombia and Japan, most *H. pylori* strains contain the more pathogenic *vacA* allele types [168]. *In vivo* experimental studies have corroborated the association, reporting mucosal injury and gastric inflammation after the administration of large quantities of VacA into the stomachs of mice [169] and demonstrating, using isogenic wild-type and *vacA*-null mutant strains that VacA contributes to severe gastritis in gerbils [170].

Monomeric 88 kDa VacA molecules are secreted into the extracellular space [171] via type V secretion [172] where they self-assemble into water-soluble oligomeric structures to form anion-selective membrane channels. However, VacA can also remain associated with the bacterial membrane as a biologically active molecule and can be taken up by the host cell in a contact-dependent mechanism [173]. The mature form can undergo specific proteolytic cleavage to yield the functionally different subunits, p33 and p55 [174]. The p33 domain contains a hydrophobic sequence that is involved in pore formation [175, 176] and vacuolating cytotoxin activity [177], whereas the p55 fragment contains cell-binding domains [178]. VacA can bind multiple epithelial cell surface molecules, including the transmembrane protein receptor-type tyrosine protein phosphatase- ζ (PTPRZ1) [179], fibronectin [180], EGFR [181], CD18 on T-cells [182] as well as various lipids [183] and sphingomyelin [184].

Besides its ability to induce the vacuolation of epithelial cells *in vitro*, VacA can also stimulate apoptosis (Figure 8), a process restricted to the more pathogenic allelic combination s1m1 [185]. The p34 VacA subunit modulates mitochondrial membrane permeability [186] by a mechanism dependent on toxin channel activity, ultimately resulting in cytochrome *c* release and the subsequent activation of caspase 3 [187]. Since VacA mutants that are defective in forming membrane channels fail to elicit cytochrome *c* release, it seems likely that VacA-mediated alterations of mitochondria are based on the formation of VacA channels in the mitochondrial membrane [188]. In stark contrast to VacA, CagA exhibits antiapoptotic features [123], leading to the hypothesis that these two key virulence factors may

have antagonistic functions. Indeed, CagA has been shown to inhibit VacA-induced apoptosis [189] and reduce vacuolation of epithelial cells, while VacA can inhibit CagA-mediated cellular elongation [190]. Within the latter scenario, active VacA exerts its inhibitory impact by interfering with signaling pathways known to be crucial for cell scattering and elongation, for example, the EGFR pathway [191]. The opposing behavior of these two key virulence factors is even more fascinating because *cagPAI*-positive strains are most likely to possess the more toxic s1 forms of VacA, whereas *cagPAI*-negative strains generally harbor nontoxic s2 forms. This is not due to genetic linkage as the gene loci are not close to each other. The antagonistic properties of VacA and CagA could represent a strategy to protect the ecological niche of *H. pylori* against its own bacterial virulence factors, which are initially needed for colonization but later have detrimental consequences for the human host. Moreover, the immunosuppressive properties of VacA may play an important role in enabling *H. pylori* to persistently colonize the human host.

12.5. Additional Virulence Attributes. Besides *cagPAI*, CagA, and VacA, other bacterial virulence effectors are known to trigger *H. pylori*-mediated gastric disease. The duodenal ulcer-promoting gene A (*dupA*), encoding a VirB4 ATPase homolog, is associated with an increased risk of developing duodenal ulcer but a reduced risk of gastric atrophy and gastric cancer [192]. Recent work indicates this could be due to *dupA*-mediated induction of proinflammatory cytokine secretion by mononuclear cells [193].

Another gene implicated in peptic ulcer disease is *iceA1*, albeit with considerable geographic differences in expression [194]. The gene encodes a CATG-recognizing restriction endonuclease with significant sequence homology to *nlaIIR*, an endonuclease of *Neisseria lactamica* [195, 196], and is induced by bacterial adherence to the gastric epithelium [197].

The Hop protein family member, bacterial outer membrane protein OipA (HopH) has also been identified as a potential disease-promoting factor. Like *vacA*, the *oipA* gene is present in all *H. pylori* strains, but its expression is modified by phase variation, caused by variable numbers of CT dinucleotide repeats in the 5' region. OipA was originally identified as a proinflammatory response-inducing protein, but may also serve as an adhesin [25]. Its expression correlates with increased *in vitro* and *in vivo* production of IL-8 [198] and, more recently, OipA has been shown to be involved in the activation of the focal adhesion kinase (FAK) and cytoskeletal reorganization, resulting in an altered morphological host cell phenotype [199]. Other adhesins of the Hop protein family such as SabA (HopP) and BabA (HopS) are also categorized as bacterial virulence factors since they mediate bacterial adherence to the host and thus influence pathogenesis.

12.6. Host Cell Determinants of *H. pylori* Pathogenesis. It has become apparent that not only the pathogen but also host

genetics play an important role in determining the clinical manifestation of *H. pylori* infections. Indeed, host genetic polymorphisms affecting expression levels of important genes involved in pathogenicity have been demonstrated to influence susceptibility and severity of *H. pylori* infection. In general, genetic polymorphisms in proinflammatory genes tend to increase the risk of gastric cancer, as demonstrated for IL-1, a potent proinflammatory cytokine and the most prominent inhibitor of gastric acid secretion [200]. IL-1 is encoded by a gene cluster containing the polymorphic IL-1B (IL-1 cytokine-encoding gene) and IL-1RN (IL-1 receptor antagonist encoding gene) encoding genes. Several polymorphisms, such as IL-1B*-31C, lead to the expression of large quantities of IL-1 β and a subsequent reduction in acid secretion [201]. Reduced acid secretion is linked to corpus-predominant colonization by *H. pylori*, which results in pangastritis formation of atrophic gastritis and thus an increased risk of gastric cancer and gastric ulcer disease [202–207]. Similar effects have been described for polymorphisms in other inflammation-associated genes, for example, the genes encoding tumor necrosis factor alpha (TNF- α) and IL-10. Distinct TNF- α polymorphisms lead to increased TNF- α expression, which influences, in concert with IL-1, gastrin production and thus acid production by parietal cells [208]. In addition to alterations in acid production, cancer patients carrying the IL1B-511T/T genotype show significantly higher methylation levels of specific genes than patients with other genotypes. This leads to the assumption that the L1B-511T/T allele is associated with enhanced hypermethylation of multiple CpG island loci, which might contribute to an increase in the risk of gastric cancer in *H. pylori*-infected individuals [209]. Similar to specific IL-1 genotypes, these TNF- α polymorphisms are, therefore, strongly linked to *H. pylori* infection and increased risk of gastric cancer [202, 210, 211]. In addition, specific IL-10 haplotypes lead to higher cytokine expression levels, thereby, shifting the balance towards an anti-inflammatory host cell [202, 212–214]; this is associated with the colonization of more virulent *H. pylori* strains [215]. By contrast, specific IL-10 haplotypes actually induce lower IL-10 expression levels, favoring proinflammatory responses and an associated increased risk of gastric cancer. Polymorphisms in other gene types may also influence *H. pylori*-induced disease; for instance, a specific allelic variant of the TLR-9 promoter gene sequence creates a potential NF- κ B binding site that increases the transcriptional activity of the gene [216]. Since altered NF- κ B activation is associated with premalignant gastric changes, this genotype could also be involved in *H. pylori*-mediated gastric cancer. Moreover, genes encoding IL-8 and NOD1 have also been shown to be associated with *H. pylori*-induced duodenal ulcer and gastritis [217]. Interestingly, the number of polymorphisms seems to influence the clinical outcome dramatically. Whereas single polymorphisms of genes involved in proinflammatory responses may increase the risk of cancer development only two- to threefold, the presence of multiple genotypes increases the risk further [205, 218].

13. Beyond Peptic Ulceration and Gastric Cancer

13.1. MALT Lymphoma. All *H. pylori*-infected persons have a significantly increased risk for the development of gastric MALT lymphoma [7]. Accordingly, the majority of MALT lymphoma patients are *H. pylori* positive [219]. In very rare cases, a monoclonal population of B-cells arises from this mucosal tissue and proliferates to form a MALT lymphoma via chronic T-cell driven antigenic stimulation. The incidence of MALT lymphoma in *H. pylori*-infected patients is estimated to occur in less than 1% of *H. pylori*-positive subjects [220], but due to diagnostic controversies, based on difficult histological interpretations, and the rarity of this disorder, no exact figures are known. In contrast to gastric cancer, where the “point of no return” often abolishes successful treatment of adenocarcinoma via the eradication of *H. pylori*, the eradication of the bacteria in MALT patients can lead to complete remission in 60%–80% of patients with stage 1 low-grade gastric MALT lymphoma [221–224]. However, 10–35% of patients in complete remission after *H. pylori* eradication showed recurrent disease, necessitating the implementation of mandatory long-term follow-up examinations [225]. Recurrence in specific patients could be due to the presence of at [11; 18] (q21; q21) translocation, which is associated with *API2-MALT1* fusion. *API2* is involved in apoptosis, and the latter resembles a caspase-like protein. The gene fusion leads to the suppression of apoptosis, and several studies have shown that MALT-lymphoma patients with this translocation do not or only rarely respond to *H. pylori* eradication [226, 227]. Despite these caveats, *H. pylori* eradication has been designated as the first choice treatment in the Maastricht III Consensus Report [228].

13.2. GERD. The development of gastroesophageal reflux disease (GERD) has long been considered to be independent of *H. pylori* as it occurred at the same frequency in *H. pylori*-positive and -negative patients [229]. An intriguing, albeit controversial, role for *H. pylori* has emerged from observations that the bacterium's prevalence was low in GERD patients [230], and that the incidence of GERD increased after *H. pylori* eradication [231], suggesting that the bacteria play a protective role. Moreover, *H. pylori*-induced corpus gastritis has been shown to reduce acid secretion and thus prevent patients from contracting GERD [232]. Conflicting evidence exists, however, demonstrating that *H. pylori* eradication has no impact on either the new cases of GERD [233] or the worsening of preexisting cases when treatment has been withdrawn during disease remission. This inverse correlation between *H. pylori* and GERD, if it exists, warrants further study before sound scientific conclusions can be made.

13.3. Extra-Gastrointestinal Disease. A putative role for *H. pylori* in the development of idiopathic thrombocytopenic purpura (ITP) was first described by Gasbarrini and colleagues [234]. Subsequent studies showed that platelet counts

in patients with ITP returned to normal levels after the eradication of *H. pylori* [235, 236]. In addition, anti-CagA antibody titers have been shown to be significantly decreased in patients responsive to *H. pylori* eradication therapy in comparison to nonresponsive patients, implicating CagA in ITP pathogenesis [237].

A number of studies have also demonstrated a link between the pathogen and iron deficiency anaemia (IDA), another extragastrointestinal disease. The prevalence of *H. pylori* was highly increased in patients with unexplained IDA, and patients showed normal haemoglobin levels after *H. pylori* eradication therapies [238]. Interestingly, despite normal serum transferrin and iron levels, soluble transferrin receptor (sTFR) was significantly elevated in *H. pylori*-infected children, suggesting that sTFR is a more reliable indicator of iron status than serum iron or ferritin.

Similar to GERD, a negative correlation between *H. pylori* and disorders such as asthma, allergy, and atopic disease has been postulated. In general, *H. pylori* prevalence and asthma as well as allergic diseases show an inverse association [239]; in children not predisposed to atopy, an inverse correlation between *H. pylori* and eczema has been observed [240]. Furthermore, due to the observations that diminished exposure to microbes during childhood leads to an increase of atopic disease [241], the debate regarding whether *H. pylori* should always be eradicated upon diagnosis has been heightened.

14. Diagnosis and Treatment

Diagnostic tests for *H. pylori* are generally divided into two categories: invasive and noninvasive. Invasive tests comprise the histological examination of gastric specimens. Noninvasive tests are based on peripheral samples such as blood, breath, stools, urine, and saliva, in order to detect antibodies, bacterial antigens, or urease activity. The choice of a specific test always depends on local experience and clinical settings, but usually a combination of two methods is often recommended since, for example, the detection of *H. pylori*-specific antibodies does not ultimately reflect a current infection.

Although *H. pylori* is sensitive to a wide range of antibiotics *in vitro*, they all fail when applied as monotherapy *in vivo*. Therefore, a combined therapeutic strategy is used, usually including two antibiotics (clarithromycin, combined with amoxicillin or metronidazole) and either a bismuth compound or a proton pump inhibitor (PPI). Rarely, quadruple therapies are used in which the bismuth compound and PPI are used in combination with two antibiotics. The use of these drugs has resulted in effective therapies, with eradication rates over 80%. During the past several years, however, resistant bacteria have been detected constantly [242, 243], leading to the search for alternative drugs and treatment strategies.

In the past decade, much effort has been devoted to the development of vaccination strategies. Based on the successful elimination of *Helicobacter felis* after mucosal immunization of mice with urease [244], the focus of much

research has been the induction of a humoral or Th2-driven immune response. To date, however, effective vaccination has only been observed in animal models and no human vaccine trial has been successful [245]. The failure to replicate the success of the vaccine in humans may be due to differences in *H. pylori*-specific immune responses or anatomical differences of the stomach. For instance, one of the main surface bacterial virulence factors, *cagPAI*, is usually switched off in mice.

Vaccines and antibiotics are not the only ways to prevent and cure *H. pylori* infection or *H. pylori*-associated disease. *H. pylori*-positive individuals infected with helminths have standard levels of *H. pylori* colonization rates and gastritis patterns, but they develop significantly less *H. pylori*-associated disease [246, 247]. These are intriguing observations that might result in low-dose administration of immunomodulating agents to *H. pylori*-positive patients, which have the same consequences as enteric helminth infections.

Another approach is the application of probiotics. There is convincing evidence that *H. pylori* is killed by *Lactobacilli* both *in vitro* and to a limited extent *in vivo* [248–250]. Furthermore, *Lactobacilli* show a positive impact on some *H. pylori* therapy-related side effects, and recent studies suggest that *Lactobacilli* supplements could be effective in increasing eradication rates [251].

15. Closing Remarks

The discovery that the world's most common bacterial infection is clearly associated with the development of severe human gastric disease signaled a medical revolution that has already significantly reduced the incidence of one major human disorder (duodenal ulcer disease) and also promises to decrease a global lethal malignancy (gastric cancer). The great leaps forward in understanding the mechanisms of *H. pylori* pathogenesis are redefining our understanding of bacterial ecology and homeostasis; however, we are still a long way away from completely understanding how *H. pylori* is associated with the host and the development of disease. Elucidating this conundrum faces a number of challenges that require a combination of global and more focused, in-depth analyses. Monitoring the epigenetic changes occurring during infection, alongside more detailed analyses of the *H. pylori*-induced adaptive and innate immune responses may help to decipher the reasons for the failure of current vaccines. Given the increased incidence of antibiotic resistance, discovery of so far unknown bacterial virulence factors may potentially facilitate the development of new drugs. Moreover, in light of accumulating data showing that *H. pylori* infection could be beneficial for humans, we may need to rethink the commonly used medical approaches to treat *H. pylori* infections.

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