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# Helicobacter pylori Recent advances in the study of its pathogenicity and prevention

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## Abstract

*Helicobacter pylori* has acquired great importance during the last two decades, after being recognized as an important pathogen that infects a great portion of the human population. This microorganism is recognized as the main causal agent of chronic gastritis and duodenal ulcers, and it is associated with the subsequent development of gastric carcinoma. The pathogenic mechanisms of *H. pylori* and their relation to gastric ailments have not been clearly defined. However, at present it is well established that urease, vacuolating cytotoxin VacA, and the pathogenicity island (*cag* PAI) gene products, are the main factors of virulence of this organism. Thus, individuals infected with strains that express these virulence factors probably develop a severe local inflammation that may induce the development of peptic ulcer and gastric cancer. The way the infection spreads throughout the world suggests the possibility that there are multiple pathways of transmission. Due to the importance that *H. pylori* has acquired as a human pathogen, laboratories worldwide are attempting to develop a vaccine that confers long-term immunological protection against infection by this microorganism. Hence, the objective of this review is to present the most relevant findings of the biology of *H. Pylori* and its interaction with the human host. The full version of this paper is available too at: <http://www.insp.mx/salud/index.html>

Key words: *Helicobacter pylori*; gastric cancer; vaccination

## Resumen

*Helicobacter pylori* ha adquirido gran importancia durante las últimas dos décadas, al ser reconocido como un importante patógeno que infecta una gran porción de la población humana. Este microorganismo es reconocido como el principal agente que causa la gastritis crónica y la úlcera duodenal, además de que se ha asociado con el subsecuente desarrollo del carcinoma gástrico. Los mecanismos patogénicos de *H. pylori* y su relación con los padecimientos gástricos no se han definido en forma clara. Sin embargo, actualmente está bien establecido que la ureasa, la citotoxina vacuolizante VacA y los productos de los genes de la isla de patogenicidad (*cag* PAI) son los principales factores de virulencia de este organismo. Así, los individuos infectados con cepas que expresan dichos factores de virulencia, probablemente manifiesten una marcada inflamación local que podría inducir el desarrollo de úlcera péptica y cáncer gástrico. La manera como la infección se propaga a nivel mundial sugiere la posibilidad de múltiples vías de transmisión. A consecuencia de la importancia que *H. pylori* ha adquirido como patógeno humano, los laboratorios del mundo se esfuerzan para desarrollar una vacuna que confiera protección inmunológica de larga duración contra la infección por este microorganismo. El objetivo de esta revisión es presentar los hallazgos más relevantes sobre la biología de *H. pylori* y su interacción con su huésped humano. El texto completo de este artículo también está disponible en: <http://www.insp.mx/salud/index.html>

Palabras clave: *Helicobacter pylori*; cáncer gástrico; vacunación

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*Helicobacter pylori* was probably already established in the human stomach at least 100 000 years before the beginning of the human exodus from Africa and thereafter it followed humans as an unknown pathologic agent.<sup>1</sup> However, in 1982 this bacterium was removed from the group of the unrecognized etiologic agents when it was discovered and isolated through accidental extended incubation of pure culture from human gastric mucosa biopsies.<sup>2,3</sup> These events and subsequent studies of its genome sequence, physiology, adaptation to a restricted ecological niche, and possible antigenic variation mechanisms, have changed radically the gastroenterology and microbiology knowledge. *H. pylori* is a microaerophilic, Gram-negative, slow-growing, spiral-shaped, and flagellated bacteria. To date, *H. pylori* is a well-recognized pathogen that chronically infects the stomachs of up to 50% of the world's human population, and that has been associated with serious diseases with significant morbidity and mortality.

#### Association between *H. pylori* infection and disease

As with most infectious diseases, *Helicobacter* associated gastritic disease is influenced not only by the nature of the infecting strain but also by the host and environmental factors. *H. pylori* infection is confined to the gastric mucosa, where it stimulates a marked local inflammation and a systemic immune response.<sup>4</sup> Over the long term, susceptible individuals are at risk of developing complications. Gastric inflammation leads to atrophy and metaplasia, conditions strongly associated with stomach cancer.<sup>5</sup>

Since *H. pylori* research has developed rapidly, it is now possible to establish a conclusive link between *H. pylori* and different forms of gastroduodenal abnormalities.<sup>6</sup> Experiments using human volunteers and animal models have substantiated the strong direct relationship between *H. pylori* and gastritis. Two well-described studies of self-inoculation with consistent temporality have been performed.<sup>7-9</sup> Although none of the experiments using humans volunteers have linked *H. pylori* infection with peptic ulcer disease of the stomach and duodenum, several laboratories have provided a mass of strong circumstantial evidence.<sup>10, 11</sup> Similarly, other studies have established a strong relationship between *H. pylori* infection and gastric adenocarcinoma, although the nature of the relationship is still controversial.<sup>12-15</sup> Moreover, there is a strong association between *H. pylori* infection and low-grade gastric lymphoma arising from mucosa-associated

lymphoid tissue.<sup>16</sup> *H. pylori* infection also has been associated with dyspepsia and non-ulcer dyspepsia, although this association is still unresolved.<sup>17</sup> Taken all together, *H. pylori* infection in humans represents a serious public health concern. Each year, there are at least 7 million patients around the world who are affected by these diseases, leading to hundreds of thousands of deaths.<sup>18</sup> The World Health Organization classifies this bacterium as a Type 1 carcinogen.<sup>19</sup>

Although *H. pylori* infection is minimally invasive, metaplastic gastric tissue may also spread to other parts of the alimentary tract such as the duodenum,<sup>20</sup> the proximal esophagus,<sup>21</sup> the distal esophagus,<sup>22</sup> Meckel's diverticulum,<sup>23</sup> and the rectum.<sup>24</sup> In addition, it has been proposed that although *H. pylori* infection is circumscribed to the gastric mucosa, it could conceivably produce lesions remote to the primary site of infection, by altering levels of systemic inflammatory mediators.<sup>25</sup> Several non-gastrointestinal tract diseases associated with *H. pylori* infection include diabetes mellitus, thyroiditis, heart diseases, rheumatoid arthritis, dermatological disorders, hepatic encephalopathy, childhood anemia and several more. Nevertheless, the hypotheses that have linked *H. pylori* infection to this variety of non-gastrointestinal tract diseases have not yet been fully substantiated.<sup>26,27</sup>

Although *H. pylori* infection may cause other diseases in addition to gastritis, there are no clinical consequences whatsoever in 60 to 70% of infected humans. This suggests that *H. pylori*-associated disease is influenced not only by the pathogenic nature of the infecting strain but also by other factors. Genetic variability in host factors such as gender,<sup>28,29</sup> blood group antigens,<sup>30</sup> human lymphocyte antigen type, and the age of the host when the infection was acquired, may play a primary role in determining different clinical outcomes.<sup>31</sup>

Environmental factors may also play a role in the outcome of the infection. Socioeconomic class, which affects living conditions and sanitation, may increase the risk of exposure to the bacterium.<sup>32,33</sup> Tobacco smoking increases the risk of duodenal ulceration for patients infected with *H. pylori*.<sup>34</sup> All these environmental determinants may undergo synergistic or antagonistic interactions with *H. pylori* to render different clinical outcomes of infection. Similarly, dietary factors including high salt and low antioxidant intake are risk factors for gastric carcinoma in and of themselves.<sup>35-37</sup> However, a vegetable and fruit diet exerts a protective influence against this disease.<sup>38</sup>

Therefore, not everyone infected with pathogenic strains is expected to develop this condition.

## Epidemiological features of the infection

Although *H. pylori* infection is chronic and endemic, it has distinct epidemiological features.<sup>39</sup> The prevalence of the infection can vary widely between and within population groups. The prevalence of infection in third world countries seems to be nearly universal, beginning in early childhood and increasing with advancing age. Most children are infected in the first few months of life. By the age of five, as many as 50% are already infected, and by the age of 40, 90% are infected.<sup>40</sup> Prevalence rates are lower in developing countries with better industrialization, public health, and living conditions. Nevertheless, the rate is still several times higher than that found in developed countries, where the incidence of *H. pylori* infection is found in approximately 30% of the adult population.<sup>1</sup>

*H. pylori* incidence has become less frequent in developed countries over recent years, which reflects a so-called cohort effect. The rate of decline may be as high as 26% per decade,<sup>41</sup> and only 0.4% of uninfected adults acquire *H. pylori* each year.<sup>18</sup> In children, however, the incidence may be higher, which is consistent with the notion that *H. pylori* infection is predominantly acquired in childhood. Nevertheless, even in children, the prevalence of infection seems to be diminishing over time. This is a worldwide phenomenon that is likely due to improvements in nutrition, drinking water, and introduction of antibiotics. Moreover, declining family size reduces the opportunity for transmission and increases the age of acquisition.

To date, almost all epidemiological studies have shown an inverse relationship between *H. pylori* infection and socioeconomic status.<sup>32</sup> In the United States, non-Hispanic whites exhibit lower prevalence of infection than do African-Americans and Hispanics.<sup>26,42,43</sup> Variations in *H. pylori* prevalence between different racial and ethnic groups have also been observed in other countries, such as Taiwan and Belgium.<sup>44,45</sup> Although the reasons for these differences are not well understood, socioeconomic status during childhood is thought to play a significant role.<sup>18</sup> A genetic predisposition to infection is also feasible. Studies of monozygotic and dizygotic twins show that genetic effects influence the acquisition of *H. pylori* due to great similarities within the monozygotic pairs.<sup>46</sup> In addition, men consistently have a higher prevalence rate than that of women across all strata of race/ethnicity, age, education, and income.<sup>26,47,48</sup>

Exactly how the organism is transmitted from the stomach of one person to that of another remains unclear. The most accepted hypothesis is that the organism is transmitted through close personal contact and

that the human being is the only significant reservoir of infection.<sup>49</sup> The detection of the bacterium in saliva, dental plaque, diarrheal stools, and vomit implicates oral-oral or fecal-oral transmission as possible routes of infection. Contaminated water and raw vegetables could be a source of *H. pylori* in the fecal-oral transmission pathway.<sup>50</sup> Iatrogenic spread, through the use of improperly cleaned endoscopic equipment, has also been proposed as a possible route of infection.<sup>51</sup> There is also the possibility of zoonotic transmission because *H. pylori* has also been found in some domestic animals who have close human contact, such as cats, but at present, the risk of infection from them appears insignificant.<sup>52</sup> Nevertheless, the demographic, environmental and zoonotic factors may play a role in the spread of *H. pylori* around of the world.

## Relevance of strain types of *H. pylori* in the outcome of infection

Virulence is concept intimately linked to disease and is usually measured in terms of morbidity and mortality. Survival and multiplication are clearly the microbe's priorities, while disease is simply a manifestation of the complex interactions required to accomplish these goals. Thus, virulence determinants include all the factors contributing to the evolutionary success of the parasite, as well as to the development of the disease in the host. There are many virulence factors of *H. pylori* that contribute in dissimilar ways to gastric mucosal damage; among them are factors known to be required for the colonization and survival of *H. pylori* in the human stomach. These factors are urease and flagella that are expressed by all *Helicobacter* species. *H. pylori* manufactures large amounts of urease that helps to break down urea into carbon dioxide and ammonium, which neutralizes the acid during colonization of the stomach. The protection of *H. pylori* by this enzyme also induces apoptosis of gastric cells *in vitro* and inhibits gastric somatostatin release in animals, which could have consequences for the physiology of digestion in general.<sup>53</sup> Flagellar motility of *Helicobacter* species has been shown to be essential for successful colonization of the host. Flagella allow the bacterium to swim across the viscous gastric mucus and reach the more neutral pH underneath the mucus. This property also enables the bacterium to resist the muscular contractions that regularly empty the stomach. Mutants of the urease<sup>54</sup> or flagellar structure genes<sup>55</sup> are defective in colonization in animal models of infection.

In analogy with other enteropathogens, the adhesion to the intestinal epithelium is a critical initial step

in enteric colonization. The adhesion process of certain strains of *H. pylori* can stimulate pedestal formation and develop attaching-effacing lesions, similar to those produced by a special class of enterovirulent *Escherichia coli* cells.<sup>56</sup> It has been shown that several putative adhesins mediate the attachment of *H. pylori* to gastric epithelial cells.<sup>57-59</sup> This suggests that this bacterium could use several adherence mechanisms to establish contact with the surface of the epithelial cells. Nevertheless, despite the importance of the adhesins in colonization and virulence, they have not been proven to be essential for *in vivo* survival of *H. pylori*.<sup>60</sup>

*H. pylori* lipopolysaccharide (LPS) has also been implicated in another aspect of the pathogenesis of *H. pylori* infection. LPS is a family of toxic phosphorylated glycolipids in the outer membrane of Gram-negative bacteria and is composed of a lipid moiety (termed lipid A), a core oligosaccharide, and a polymeric O-specific polysaccharide chain.<sup>61</sup> Although, *H. pylori* LPS shares certain biological properties with LPS of other intestinal bacteria, generally it possesses lower immunological activity. *H. pylori* LPS is able to induce toxic lethality in animal models<sup>62</sup> and the secretion of cytokines by human monocytes.<sup>63,64</sup> However, this induction is weaker than that of other tested bacterial LPS. This lower immunological activity of *H. pylori* LPS is probably a result of its unusual acylation and phosphorylation pattern of lipid A.<sup>63</sup>

*H. pylori* LPS may have an important role in autoimmune-mediated damage in the gastric mucosa. The structure of the LPS O-specific chain in different *H. pylori* strains may mimic Lewis blood group antigens in structure. As these antigens are present in the normal human gastric mucosa, the expression of Lewis antigens on the bacterial surface may camouflage the bacterium and may contribute to its survival in the stomach.<sup>61</sup> Alternatively, during infection, highly conserved immunogenic molecules expressed by infectious pathogens may act as a trigger for the induction of humoral and cellular immune responses that cross-react with host cellular antigens in a mechanism known as antigenic mimicry.<sup>65</sup> Antibodies against *H. pylori* Lewis antigens have been found to cross-react with various components of the gastric mucosa. The gastric H<sup>+</sup>, K<sup>+</sup>-ATPase is a major autoantigen in *H. pylori*-associated antigastric autoimmunity.<sup>66</sup> Anti-Lewis<sup>Y</sup> antibodies induced by *H. pylori* against Lewis<sup>Y</sup> epitopes of the beta chain of this proton pump involved in acid secretion may promote the development of atrophic gastritis.<sup>65,67</sup>

On the other hand, of the molecules that are actively transported to surrounding tissues, the most important is the vacuolating toxin (VacA), which is the

major toxin secreted by *H. pylori*.<sup>68</sup> The active cytotoxin is produced by approximately 50% of *H. pylori* clinical isolates,<sup>69</sup> and its presence is associated epidemiologically with tissue damage and peptic ulcers.<sup>70</sup> VacA is initially translated as a 140 kDa protoxin, which subsequently undergoes both N-terminal and C-terminal processing to yield a 95 kDa mature secreted monomer. This mature monomer assembles into a large oligomeric complex with hexameric or heptameric radial symmetry. The mature monomer could be cleaved proteolytically into two fragments: an N-terminal 34 kDa fragment and a C-terminal 58 kDa fragment that remain associated after cleavage.<sup>71</sup> The ability to induce vacuoles is localized mostly but not entirely in the first fragment, whereas the second fragment is mostly involved in cell targeting.

Several mechanisms of VacA toxic activity against the host cell have been reported. It has been suggested that this toxin induces the alteration of intracellular vesicular trafficking in eukaryotic cells, leading to the formation of large vacuoles containing markers of late endosomes and lysosomes.<sup>72</sup> It also causes a reduction in transepithelial electrical resistance in polarized monolayers and forms ion channels in lipid bilayers.<sup>73</sup> Moreover, it interferes with antigen presentation to the immune system by impairing processing and maturation of the antigen by the antigen-presenting cell.<sup>72,74,75</sup> Finally, Yahiro K and colleagues reported that acidic and alkaline treatments of VacA protein were associated with an increase in its biological activity and in its binding to a 250-kDa receptor protein-tyrosine phosphatase  $\beta$  in AZ-521 cells.<sup>76</sup>

The vacuolating cytotoxin gene A (*vacA*), which encodes VacA protein, is present in all *H. pylori* strains, but the structure among *vacA* alleles varies, particularly in two regions.<sup>77</sup> One of these regions is the mid-region of the gene, which may be type m1 (subtype a) or m2 (subtype a or b). The other variable region of *vacA* gene is in the second half of the signal sequence, which may be type s1 (subtype a, b and c) or s2.<sup>78</sup> The final structure of *vacA* gene is a mosaic, and all combinations of signal sequence and mid-region types, including the rare s2/m1, have been reported.<sup>77,79</sup> Thus, several different families of *vacA* alleles are present in *H. pylori*, and its encoded products are associated with and without vacuolating activity *in vitro*.<sup>77,80</sup> Likewise, *H. pylori* strains containing certain types of *vacA* alleles have been associated with an increased risk for peptic ulcer disease. Atherton and colleagues showed that strains with *vacA* mid-region type m1 are more strongly associated with increased gastric epithelial damage (epithelial degeneration, mucus depletion, and microscopic erosion) than mid-region type m2. Meanwhile,



s1a strains are associated with increased mucosal neutrophil and lymphocyte infiltration *in vivo*. These results suggest that s1a/m1 strains have the most virulent allelic type activity,<sup>77,80</sup> while *vacA* s2 strains are rarely associated with ulcer disease and are uncommon in many populations.<sup>80-84</sup>

The link between the ability of a strain to induce epithelial cell vacuolation *in vitro* and peptic ulcer disease *in vivo* seems to be consistent but not invariable.<sup>77</sup> Among strains from populations in the United States, the ability of *vacA* s1a strains to induce ulcer disease was higher than that of *vacA* s1b strains. Over 90% of patients with duodenal ulcer disease harbored *vacA* s1a strains, while patients with *vacA* s2 strains were not more likely to have ulcer disease than uninfected patients.<sup>80</sup> Likewise, in a United Kingdom study population, all patients with ulcer disease had *vacA* s1 strains, and no *vacA* s2 strains were associated with this disease.<sup>83</sup> Other studies from Europe also indicate a strong correlation between the *vacA* s1 genotype and peptic ulceration.<sup>85-87</sup>

However, this correlation is not as evident in the Far East as it is in the West. Recent studies show that most *H. pylori* strains in Japan have an s1a/m1 genotype. However, regardless of the *vacA* genotype, the infection leads to peptic ulcer or gastric cancer in only a small subset of infected patients. Thus, these *H. pylori vacA* genotypes cannot be used in a reliable way to discern the risk of developing serious gastroduodenal disease in the host.<sup>78,82,88</sup>

In contrast with East Asian and Western populations, studies of the South African population have shown a high prevalence of the *vacA* s1b strains in patients with peptic ulceration. No *vacA* s1a strains were present, and 100% of patients with peptic ulceration harbored strains with *vacA* s1b allele.<sup>84</sup> The high prevalence of strains with *vacA* s1b genotype is not confined to South Africa; these strains are also widespread in Brazil and Portugal, where higher prevalence rates of peptic ulceration and gastric adenocarcinoma have been reported.<sup>86,89</sup> Additionally, in the South African population, the *vacA* s2 allele was found exclusively in the group of individuals without any pathology, consistent with observations that *vacA* s2 strains are minimally or non-ulcerogenic.<sup>80-84</sup> On the other hand, recent studies of a worldwide collection of strains indicate that subtype s1c was observed exclusively in isolates from East Asia and appears to be the major s1 allele in that part of the world. Nevertheless, whether s1c strains are phenotypically different from s1a and s1b strains remains to be determined.<sup>68</sup>

In addition to these typical differences between *vacA* alleles, there is also considerable variation among

different *H. pylori* strains with regard to the production of vacuolating cytotoxin. Thus, the vacuolating cytotoxin phenotype of an *H. pylori* strain is not only dependent on the amino acid sequence of VacA but may also be modulated by other strain-specific factors, such as the level of *vacA* transcription or the efficiency with which VacA is secreted.<sup>90</sup> The considerable variation in these determinants may be relevant to the occurrence of different clinical outcomes in *H. pylori*-infected humans.<sup>91</sup>

The high molecular weight immunodominant CagA protein encoded by the *cytotoxin associated gene A* (*cagA*), has long been one of the most intensely investigated putative virulence factors. However, its function has not been well understood. Recently, Odenbreit S. and colleagues reported that CagA is tyrosine-phosphorylated and induces changes in the tyrosine phosphorylation state of distinct proteins in gastric epithelial cells.<sup>92</sup> The *cagA* gene is present in about 50-70% of *H. pylori* strains<sup>93</sup> and virtually all of them produce a detectable local and systemic CagA-antibody response in the human host. Several studies have reported that the *cagA* status is highly associated with the pathogenicity of *H. pylori* strains.

These features make it easy to correlate the *cagA* phenotype of the infective strains with a particular gastroduodenal disease by a regular blood test.<sup>94</sup> The clinical isolates of *H. pylori* have been grouped into two broad families defined as type I and type II on the basis of whether they have the entire pathogenicity island (*cag* PAI), express the CagA protein, and secrete an active vacuolating cytotoxin VacA.<sup>93,95,96</sup> Type I strains are positive for all these characteristics. In contrast, type II strains do not express CagA even though they have the gene, possess the *vacA* gene that is silent or encoded for non-toxic but immunoreactive protein, and/or have defective secretion mechanisms.<sup>77,97,98</sup> In addition, only type I strains were able to elicit secretion of Interleukin 8 (IL-8), which is the mediator of neutrophil migration by epithelial cells *in vitro*.<sup>99,100</sup> A number of epidemiological studies have shown that there is a strong correlation between infection with type I strains and occurrence of severe gastroduodenal diseases, whereas type II strains with attenuated virulence do not induce dramatic change in the gastric mucosa.<sup>97,101</sup>

Thus, *H. pylori* strains that express CagA cause more extensive inflammation of the gastric mucosa,<sup>99,100,102</sup> and infections with these strains have been reported to be more likely to result in peptic ulceration,<sup>97,98</sup> atrophic gastritis<sup>101</sup> and gastric adenocarcinoma.<sup>102,103</sup> These epidemiological studies are supported by experiments in animal models, where only the type

I strains promote gastric damage similar to that observed in humans.<sup>71,104</sup>

To date, studies have shown that CagA is one of the proteins produced by the pathogenicity island (*cag* PAI). Recent studies using isogenic mutants strains lacking the *cagA* gene have shown that IL-8 induction may not be directly due to CagA protein but to the products of *cag* PAI genes.<sup>95,105,106,107</sup> *cag* PAI is a 40 kilobase segment of DNA containing over 31 genes inserted into the chromosomal glutamate racemase gene. The presence in *cag* PAI of the short repeated sequences similar to that found in the insertion sequence 605 (IS605), and its different proportion of G+C in comparison with the chromosomal DNA, suggests that this has been acquired by horizontal transfer.

Although *cag* PAI was acquired by a relatively recent event in evolutionary time, the structure of the *cag* PAI is not identical in all strains, which indicates that this region has gone through a series of rearrangements at different points during evolution. Cesini and colleagues suggested that after the initial event of integration of the island, *cag* PAI is found as a single uninterrupted unit in the same relative position on the chromosome.<sup>58,95,108</sup> Subsequently, the interposition by one copy of the IS605 into its intermediate region has resulted in rearrangements and deletions within *cag* PAI that have led to the rise of *H. pylori* strains with varying virulence.<sup>95</sup>

Analysis of nucleotidic sequence has revealed that six of the *cag* PAI genes belong to a growing family of transporters whose subunits share extensive sequence, genetic organization, and functional similarities. At present, this secretion family is referred to as a type IV secretion system and is present in other bacteria species such as *Agrobacterium tumefaciens*,<sup>109</sup> *Bordetella pertussis*,<sup>110</sup> *E. coli*,<sup>111</sup> and *Brucella suis*.<sup>112</sup> The type IV secretion system is devoted to the transfer of a variety of multimolecular complexes across the bacterial membrane to the extracellular space or into other cells. The similarity of the *H. pylori* *cag* PAI genes with components of bacterial secretory pathways suggested that the expression of *H. pylori* virulence is triggered by contact with the host cell. Recently Odenbreit S. and colleagues provided evidences that the *cag* PAI system is involved in CagA secretion.<sup>82</sup> In this way, the bacterial protein CagA is the virulence determinant that is actively translocated across the membrane of the gastric epithelial cells.<sup>95,107</sup>

The gastric epithelium represents the first line of active defense against *H. pylori* infection. However, this tissue not only serves as barrier to exclude pathogens but also secretes a number of inflammatory mediators that initiate the host immune response to

pathogen invasion. *In vivo* and *in vitro* studies have shown that the bacterial infection induces chemokines secretion in gastric epithelial cells, such as IL-8 and GRO- $\alpha$ , MIP-1 $\alpha$ , ENA-78, and MCP-1, which have neutrophil attractant properties. It also induces inflammatory cytokines IL-1, IL-6 and TNF- $\alpha$ , which up-regulate the expression of IL-8 in epithelial cells.<sup>67,105,113</sup> This epithelial proinflammatory cytokine/chemokine response is particularly important because it serves to amplify and spread the primary pathogenic signal that leads to a rapid mobilization of phagocytic cells to the sites of invasion. Proinflammatory cytokine response may be protective, but when chronically activated, it also disrupts the function and the integrity of the gastric epithelium. The infiltration of T-cells and basophils is activated by IL-8. However, neutrophils appear to be its major target.<sup>99</sup> Activated neutrophils may be helpful, but they may also contribute to surface epithelial layer damage by leakage of potent intracellular enzymes and reactive products. Cytokines produced by infiltrated mononuclear phagocytes within the mucosa produce additional stimulation that induces gastric degeneration.<sup>113</sup> Thereby, IL-8 appears to play a key role in the initiation of the local inflammatory and immune response that may contribute to the more serious sequels associated with *H. pylori* infection.

The importance of certain predicted coding regions of the *cag* PAI in the elicitation of chemokine response has been evaluated by mutations that suppressed or reduced the induction of IL-8 secretion by epithelial cells. These studies have shown that isogenic *cagA* mutants do not affect the expression of VacA and elicit IL-8 secretion to the same degree as does the wild-type parent strain.<sup>99,114,115</sup> However, mutant strains with disruption of many other genes in the *cag* PAI suppress their ability to stimulate IL-8 production.<sup>95,106,107,116,117</sup>

Cesini and colleagues demonstrated that mutants in *cagE*, *cagG*, *cagH*, *cagI*, *cagL*, and *cagM* genes lost their ability to induce IL-8 and blocked the tyrosine phosphorylation of CagA protein, while *cagN* mutants were not affected.<sup>95, 116</sup> Knockout of *cagE* gene affected the activation of the immediate early response transcription factor nuclear factor  $\kappa$ B (NF- $\kappa$ B) in a gastric epithelial cell line.<sup>118</sup> This protein, which is activated upon stimulation by a large variety of pathogenic agents, is present in virtually all cell types and plays an integral role in regulating the human immune response.<sup>119</sup> The active form of NF- $\kappa$ B is located in the nucleus of the cell, where it activates transcription of target genes, including those encoding IL-1, IL-6, IL-8, and TNF- $\alpha$ .<sup>118</sup>

Glocker E. and colleagues investigated the role of other *cag* PAI genes in NF- $\kappa$ B activation.<sup>120</sup> They re-

ported that while two genes (*cagF* and *cagN*) are not required for transcription factor activation, six genes (*cagE*, *cagG*, *cagH*, *cagI*, *cagL*, and *cagM*) are necessary, since isogenic *H. pylori* strains carrying mutations in these genes no longer induce NF- $\kappa$ B activity. Additionally, Keates S. and colleagues determined that gene products of the *cag* pathogenicity island are required for maximal activation of mitogen-activated protein kinases (MAPK) in gastric epithelial cells. MAPK regulate cell proliferation, differentiation, inflammatory responses, stress, and programmed death, which may help induce gastroduodenal inflammation, ulceration, and neoplasia.<sup>121</sup> The integrity of whole *cag* PAI is also a prerequisite for efficient activation of early transcription factor AP-1, which is known for its immunostimulatory function<sup>113</sup>. Hence, these data support the view that the epithelial cytokine/chemokine response depends on multiple genes in the *cag* PAI of *H. pylori*.

#### Vaccination development for clinical use and future of the *H. pylori* infection

The discovery of *H. pylori* as the causal agent of various gastroduodenal diseases has dramatically changed the medical approach to this bacterial species. Research on this bacterium's mechanisms of virulence has advanced substantially in a period of less than 20 years. The availability of the sequence of the *H. pylori* genome and functional characterization of the genes involved in virulence have considerably increased our understanding of the molecular genetic basis for the pathogenesis of *H. pylori*.<sup>96,122</sup> Although the specific genes involved in virulence are still being determined, the completed genome sequence should make it easier to determine the participation of these genes in the wide spectrum of pathological and clinical outcomes that follow *H. pylori* infection. The participation of several of these genes has already been confirmed by genetic disruption. It is now well established that urease and VacA protein are virulence factors of this organism. The *cag* PAI genes have also emerged as other well-confirmed virulence participants.

Effective antibiotic-based therapies for eradicating *H. pylori* have been developed in recent years. At this time, the most proven effective treatment is a 2-week course of treatment called triple therapy, which involves the use of two antibiotics and either an acid suppressor or a stomach lining shield. Two-week triple therapy kills the bacteria, reduces ulcer symptoms, and prevents its recurrence in more than 90% of patients.<sup>123</sup> There is, however, an increasing problem of *H. pylori* antibiotic resistance. Antibiotics cannot be used to eradicate the infection from the whole popula-

tion, especially in developing countries where their indiscriminate use has led to the emergence of *H. pylori* resistant strains.<sup>124</sup> It is therefore predictable that although antibiotics are useful, the long-term consequence of large-scale eradication programs will be a reduction in the efficacy of antibiotic therapy. Vaccination, which has long been the most efficient medical intervention in controlling epidemiological infections, seems to represent the best alternative. Therefore, the development of a vaccine against *H. pylori* that confers long-term protective immunity is the best strategy to eradicate chronic *H. pylori* infection on a global scale and to avoid the problem of antibiotic resistance.<sup>125</sup> This possibility has been the stimulus for intense research activity. Further investigation of *H. pylori* virulence factors has provided information that is being used in the development of novel therapies and vaccines to treat and prevent *H. pylori* infection.

Although the feasibility of inducing protective immune response to *Helicobacter* by vaccination was initially demonstrated in experimental *Helicobacter felis*/murine model,<sup>126</sup> the development of a vaccine has been particularly successful in the more recent *H. pylori*/mouse models that mimics human infection.<sup>104, 127</sup> These models have permitted the testing of prophylactic and therapeutic vaccines containing different antigens, including whole inactivated cells,<sup>1</sup> bacterial lysates,<sup>128</sup> and several purified antigens. To date, researchers have identified several *H. pylori* antigens which confer protection against *H. pylori* infection or in eradicating an already established infection in the murine models, including purified VacA,<sup>104</sup> urease (and its subunits),<sup>129</sup> CagA,<sup>130</sup> heat shock proteins (HspA and HspB),<sup>1</sup> and catalase.<sup>131</sup> Therapeutic vaccination has also been successful in ferrets and Rhesus monkeys infected with *Helicobacter mustelae* and *H. pylori*, respectively.<sup>132, 133</sup>

Most of these antigens have been given orally in combination with mucosal adjuvants, which improve their low immunoreactivity.<sup>127</sup> However, the inherent toxicity of mucosal adjuvants has been the major limitation for their use as vaccines in humans. An alternative approach to circumvent this problem is the use of genetically detoxified heat labile enterotoxins of *E. coli* LTK63 or non-toxic mutant cholera toxin CT S61F.<sup>125,134, 135</sup> The challenge currently faced by several laboratories is to discover whether or not the promising results obtained in the animal model can be reproduced in humans.

A major question is how vaccination could be successful if *H. pylori* can survive for the lifetime of its human host, despite the vigorous immune response that is mounted against it. Nonetheless, studies in



progress not only allow investigators to confirm the feasibility of the vaccination to prevent and/or cure gastroduodenal diseases, but also to understand how the host immune response prevents and/or exacerbates this *H. pylori* infection. A large body of evidence has indicated that the major subset of lymphocytes involved in immunity against invading *H. pylori* is the CD4+ T-lymphocytes which are divided into Type 1 T helper (Th1) and Type 2 T helper (Th2) phenotypes.<sup>136</sup> Th1 cells produce a set of cytokines which activate macrophages and are responsible for cell-mediated protective responses. In contrast, Th2 cells produce cytokines, which are responsible for eosinophil activation, inhibition of several macrophage functions that provide phagocyte-independent protective responses, and strong antibody production, particularly IgA and IgG, which are especially active on mucosal surfaces.<sup>137,138</sup>

In the stomach, IL-12 and IL-10 production has been implicated in the selection of Th1 and Th2 cells, respectively. The relative predominance of IL-12 in the human stomach, regardless of whether it is infected or not, favors the development of a subset of helper T cells that tend to produce interferon gamma, which is associated with Th1 cells and enhanced cell-mediated immunity.<sup>127</sup> More recently, several laboratories have shown that biopsies from gastric mucosa of normal, infected, and uninfected patients, revealed no or very low levels of mRNA of the classic Th2 cytokine IL-4.<sup>137,138</sup> These studies support the notion that most infected humans have a Th1 response, which does not eradicate *H. pylori* infection. It is therefore predictable that a Th2 response would be more effective.

In contrast to natural infection, urease alone is able to induce relatively more IL-10 in animal models.<sup>139</sup> These findings support the notion that vaccines, including whole-cell vaccines and native or recombinant urease or urease subunits, may be effective due to their ability to alter the selection of T-cell subsets.<sup>137</sup> Additionally, the use of non-toxic LTK63 or CT S61F for oral vaccination induces a relatively greater Th2 cell response that can cure and prevent infection in *H. felis*/mice models.<sup>128,129,135,139</sup> Thus, with the discovery of the appropriate formulation and routes of administration, it is possible that vaccination could trigger an immune response that differs substantially from that induced by natural infection with *H. pylori*. Undoubtedly, the continuous efforts of laboratories around the world to develop a vaccine and therapeutic approaches may soon bring an end to the long evolutionary relationship between humankind and *Helicobacter pylori*.

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