Continued medical education

Gastric carcinogenesis

William Otero Regino, MD,1 Martín A. Gómez,2 Denny Castro.3

1. Associate Professor of Medicine, Gastroenterology unit, Universidad Nacional de Colombia, Gastroenterologist at the Clínica Fundadores de Fundación Hospital San Carlos and at the Clínica Carlos Lleras Restrepo, Bogotá, Colombia.
2. Professor of Medicine, Gastroenterology Unit, Universidad Nacional de Colombia, Hospital El Tunal, Gastroenterologist at the Hospital El Tunal, Fundacion Hospital San Carlos y Clínica Carlos Lleras Restrepo, Bogotá, Colombia.
3. General Director and Director of Graduate Studies of Gastroenterology at the Centro de Control de Cáncer Gastrointestinal “Dr Luis E Anderson” San Cristobal, Estado Táchira Venezuela.

Received: 02-02-09
Accepted: 12-08-09

Summary
Gastric cancer is second among cancers as a cause of death. More than 90% of all gastric cancers are adenocarcinomas whose principal cause is Helicobacter pylori. Although H. Pylori is a necessary condition, it is not a sufficient condition since only 1-2% of those infected develop gastric cancer. There are multiple factors besides H. Pylori infection involved in the etiology of this cancer. They include genetic factors related to the individual and environmental factors. Although the ways in which H. Pylori participates in this carcinogenesis are not completely clear, two different mechanisms are involved. H. Pylori infection induces persistent inflammation accompanied by hyperproliferation of cells, and it causes damage to DNA from free radicals in which progenitor cells from the bone marrow participate. These cells could be the “stem cells” of gastric cancer. The second path involves the direct action of proteins from H. Pylori on gastric cells. Among the genetic factors involved there is evidence that IL-1B, TNF, IL-8, and INF gamma e IL-10 polymorphisms, among others, induce strong inflammatory responses which are associated with higher risks of gastric cancer.

Key words
Helicobacter pylori, carcinogenesis, stem cell, polymorphisms

Gastric cancer (GC) is heterogeneous and highly prevalent globally. In 2002 it was estimated that there were 900,000 new cases and 700,000 deaths (1). Overall, it is the fourth most common cancer and second leading cause of cancer deaths (2), explaining 10% of them (3). In Japan (4) and Colombia (5) it is the leading cause of cancer death. In Colombia its incidence is approximately 10 times higher than in the USA (5). Over 90% of GCs are adenocarcinomas (6,7). The rest are less frequently occurring tumors such as lymphomas, gastrointestinal stromal tumors (GIST) and carcinoid tumors (7). The primary etiologic agent of distal or non-cardial GCs and MALT lymphoma is Helicobacter pylori (H. pylori) (8). Gastric cancer develops in 1 to 2% of the people infected with these bacteria, while low grade malignancy MALT lymphoma occurs in 0.7 to 0.8 per 100,000 individuals. This is in contrast to the USA, where 1 case per 30,000 to 80,000 individuals occurs, and to Italy where up to 13 cases occur per 100,000 individuals (9).

According to the classification of Finnish pathologists Jarvi and Lauren, GCs are divided into two histological types: intestinal and diffuse (10,11). They have clear differences in terms of epidemiological, histopathological, endoscopic, clinical and pathogenetic points of view (12). Most CGs are sporadic, but approximately 10% are grouped in families and 10 to 30% of these are inherited (13). The intestinal type is most frequent in sites with high GC prevalence, have better prognoses and occur more often in men over 50 years of age. However, in places with high prevalence it appears at an earlier age (6,7). In places with low prevalence or low risk, as in many developed countries, its incidence has declined in recent decades (7). This GC type appears in stomachs that have atrophic gastritis and intestinal metaplasia. There are higher numbers of cases in underdeveloped countries (6,7,14).

In contrast the diffuse type shows no geographic variation, is more common in women, often appears in young people with positive family history, has a worse prognosis and is
not associated with gastric atrophy or intestinal metaplasia (6, 14). Its incidence has not changed and even seems to be increasing (15, 16). Esophagogastroduodenoscopy, the best method for GC diagnosis, often has difficulties in the diagnosis of diffuse GC because this cancer tends to follow a submucosal pattern rather than forming exophytic masses (16, 17). The diagnosis is even more difficult when there is linitis plastica.

1-3% of all GCs are inherited. This subset represents a clearly defined clinicopathologic syndrome, known as hereditary diffuse GC (17, 18). In 30% of the families with this entity, mutations have been found in the epithelial cadherin gene (E-cadherin or CDH1) which is an encoded cell adhesion protein (19-22). The mutation is autosomal dominant with 70% penetrance, to be precise the patient has a 70% risk of suffering from GC. Women with this mutation have increased risks of lobular breast cancer (19, 22). This GC has high mortality at an early age. When diagnosed, most patients already have advanced disease and high risks of post surgical relapse (16). In individuals with CDH1 mutations, prophylactic gastrectomy is recommended. For those who do not accept this procedure, upper gastrointestinal endoscopy every 6-12 months is recommended (22).

Today, it is recommended that patients be checked for CDH1 mutations in the following situations (22):
1. Families with two or more cases of diffuse GC, one of them, diagnosed before the age of 50.
2. Families with more than three cases of diffuse GC, diagnosed at any age.
3. Isolated individuals with a diagnosis of diffuse GC before 35 years of age.
4. Isolated individuals with lobular breast cancer and diffuse GC.
5. Families with a member with diffuse GC and another with breast cancer or colon cancer with signet-cell ring. It is also recommended that families with multiple cases of lobular breast cancer with or without cases of diffuse CG be tested, since CDH1 mutations have also been detected in families with only lobular breast cancer (23).

Taking into account that most GC cases are intestinal, this article will focus on the carcinogenic pathways involved in these cases and will not deal with other tumors. The inspiration for this review came from Doctors Pelayo Correa and David Graham, two universally known figures who have contributed substantially to the understanding of this important disease.

Gastric cancer is a multifactorial disease that develops through a multi-step process that can last 20 years or more (24). Its genesis is complex and involves the participation of three main factors: environmental factors, the agent (H. pylori) and host genetic factors (6, 7.12, 14, 24, 25).

**Environmental factors**

*Diet:* Multiple observational studies have examined the association between fruit and vegetable consumption and risk of GC. Some have found that fresh fruit, vitamin C and beta carotene are associated with reduced risk of GC (26-29) although others have not found this effect (30). A Cochrane review concluded that there is no evidence that dietary supplementation with antioxidants, including vitamin C, reduces the risk of GC (31). However, no clinical trials have studied the effectiveness of vegetable and fruit consumption on GC risk. The evidence for its protective effect comes only from epidemiological studies (32).

The results of clinical trials with betacarotene and other antioxidants for prevention of GC are not consistent. In Colombia, Correa et al (33) found that a supplement of 6 mg of beta carotene daily for six years significantly increased the regression rate of gastric atrophy and intestinal metaplasia. However, long-term monitoring of patients in this trial found that the benefit of vitamin C and beta carotene disappeared with time, unlike what had happened in the eradication of *H. pylori* (34). These results correlate with the work of Blot et al in China which found that supplementation with beta carotene (15 mg), alpha-tocopherol (30 mg) and selenium 50 ugr for 5 years did not alter the mortality rates of patients with cancer of the gastric cardia nor of distal or non-cardia gastric cancer (35).

Experimentally, carotenoids (lycopene, lutein and B-carotene) and retinoids inhibit the growth of chemically induced gastric tumors in laboratory animals (36-39). It is believed that the benefits of carotenoids in GC genesis result from mechanisms such as decreased cell proliferation while simultaneously an antioxidant effect blocks free radicals, preventing oxidative DNA damage (37, 39) and induced apoptosis (40), decreases the *H. pylori* bacteria population and reduces inflammation by correcting the immune response to Th2 (rather than the ‘wrong’ answer that paradoxically is Th1) (41, 42).

**Salt:** There is evidence of increased GC risk in individuals who have a high salt intake or a high intake of foods preserved in salt (7.9). In an investigation by the World Cancer Research Foundation (WCRF) and the American Institute for Cancer Research (AICR) (43), eight studies found increased GC risk (OR 2.1 to 5.0) with salt consumption. However, four studies found no association. Experimentally salt increases gastric tumors (44, 45). High salt concentrations in the stomach produce various harmful effects on the stomach including inflammation and damage to the mucus layer.
Nitrate, nitrates and nitrosamines

Humans are exposed to two sources of nitrosamines. The first source is preformed nitrosamines present in meats, fish and other foods preserved with nitrites such as pickled, smoked, and salted food and in drinks like beer and whiskey (49). The second source is the vegetable nitrates used as additives in cured meats and cheeses (50). Nitrates in the diet can be reduced to nitrates by bacteria in the oral cavity and these reduced to N-nitroso compounds by bacteria in the stomach in reactions with amides, amino acids and amines (50). Dietary nitrates can also be reduced to nitrates by the formation of nitric oxide when inflammation is present (32, 49, 50). Increased GC risk has been found with nitrosamines formation when H. pylori infection or decrease vitamin C plasma levels are present (51). Different experimental and observational studies suggest that nitrosamine and consumption of processed foods with related substances increase the GC risk (32).

Alcohol. The association of alcohol with GC is controversial. Available data do not support the concept that this substance is associated with increased GC risk (32).

Helicobacter pylori

H. pylori was the first bacterial pathogen to be classified as a type I carcinogen by the International Agency for Research on Cancer (52). This rating was originally based on the results of three large epidemiological studies (53-55) conducted before the association was demonstrated experimentally (56, 57). This pronouncement by the IARC, without experimental evidence, was criticized by some scientists as hasty. The epidemiological studies mentioned showed that the GC risk for people infected with the bacteria ranged between 2.8 and 6 times greater than uninfected people. The EUROGAST international study also found that those infected had an increased GC risk with an OR of 6 (58). A relatively recent metaanalysis found that the risk was 2.28 for individuals with GG whose serology was positive for H. pylori and 2.87 when the microorganism was Cag A (+) (59). The studies mentioned above, and other more recent studies, have concluded that the evidence on the relationship between H. pylori and GC is unequivocal. This microorganism is the main causative agent of this tumor (60-63), however a small proportion of GCs may possibly be associated with Epstein Barr virus (64).

It is currently believed that H. pylori is responsible for more than 90% of GCs (62). Nevertheless, H. pylori infection is not sufficient to cause gastric cancer as only a minority of those infected will develop GC. Today the causal association of this infection is no longer disputed. The controversial areas now lie in the identification of the mechanisms by the bacteria produce GC and in methods for identification of individuals at high risk of developing GC.

All H. pylori are pathogens and cause chronic gastritis in all infected persons (62, 65-68). However, some organisms are more virulent than others. Chronic gastritis is asymptomatic. Different types are associated with different final clinical outcomes: antral gastritis with duodenal ulcer cases and body pangastritis/gastritis with gastric cancer or gastric ulcer cases (68-71) as shown in figure 1. The associations between these two types of gastritis and these two diseases has been recognized for over 70 years (72, 73).

The ultimate consequences of the infection depend on genetic characteristics of the host, on the characteristics of the particular type H. pylori and on external environmental factors. The two types of chronic gastritis produce different alterations in gastric physiology. In antral gastritis, there is acid hypersecretion (70, 71) which results in duodenal bulb gastric metaplasia with alteration of defense mechanisms and eventual duodenal ulcer. It has long been known that patients with duodenal ulcers, despite having antral gastritis, have reduced GC risk (62, 69, 74), whereas gastric ulcers increase the GC risk (75). In Japan, where incidence and prevalence of GC is high, the duodenal ulcer/gastric cancer ratio is less than 1 (76). In regions with low GC prevalence, including some Western countries and Southeast Asia, the ratio is greater than 1 (77, 78). In Japan, Uemura et al. (74) found that none of the patients infected with H. pylori who had duodenal ulcers developed gastric cancer during the eight years they were tracked. In contrast, 3.4% of the patients with gastric ulcers developed GC. That study also confirmed that patients with duodenal ulcers and antral gastritis usually had little or no atrophy, whereas those with gastric ulcers usually had body-antral gastritis with various degrees of atrophy. The predominant body gastritis was associated with a relative risk (RR) of 34.5 for GC (74).

The mechanisms by which H. pylori produces GC are not fully known, but today carcinogenesis may involve indirect mechanisms (permanent inflammation) and direct mechanisms represented by the action of different virulence factors of H. pylori on the gastric epithelium (76).

INDIRECT MECHANISMS

These are related to the strong inflammatory response produced in the infected stomach. It causes morphological and molecular changes in the epithelium resulting in the following sequence. Histopathologically, in 1-2% of those infected, chronic gastritis develops, followed by gastric atrophy, complete intestinal metaplasia, incomplete intestinal metaplasia, dysplasia and cancer (68, 79, 80). Atrophy,
which is usually present, may have a multifocal or diffuse pattern and may be associated with a form of metaplastic mucosa called pseudopyloric (body antralization) (81). This is also known as metaplasia expressing spasmolytic polypeptide, since these antral gastric body glands express this polypeptide which is a trefoil peptide normally present in the intestinal mucosa in normal and dysplastic cells and in neoplastic cells (82).

These hypothetical pathways suggest that chronic inflammation leads to gastric atrophy, a preneoplastic stomach lesion. Chronic inflammation causes an increase in tissue turnover with excessive cell proliferation. This can predispose to frequent mitotic errors with increased risk of mutagenesis (69, 76, 80, 82). The concurrence of cellular hyperproliferation with inflammation involves the generation of cytokines, growth factors and free radicals of oxygen and nitrogen (nitric oxide) (62, 68, 83). This favors the possibility of damage to the DNA of the gastric cells which may induce mutations in the DNA, or genes, “silencing” them at the transcription level (62, 68). Matsumoto et al found that *H. pylori* infection caused induced activation expression of the cytidine deaminase (AID) gene (84). This can predispose the p53 tumor suppressor gene to mutations (85). Harmful environmental factors such as smoking and high levels of salt in the diet further increase the GC risk, whereas diets high in antioxidants such as fresh fruit and vegetables may be protective (31).

This sequence of events is the classic paradigm of Professor Pelayo Correa’s model (12, 26, 68). Dr. Correa has the merit of having proposed the theory of gastric carcinogenesis over 30 years ago prior to the discovery of *H. pylori*. The process involves a multi-step progression from gastritis to cancer (86). Dr. Correa’s paradigm gradually became more important with the discovery of *H. pylori* as the main cause of chronic gastritis (87) (figure 2). This model has continued to evolve a number of changes that affect the natural history of infection have been identified (82). Correa posited that atrophic gastritis was multifocal, occurred throughout the whole stomach and was more frequent in geographical areas with the highest incidences of GC (88, 89). In addition, he demonstrated that populations with higher risks of GC in Colombia had higher prevalences of atrophic gastritis than those with lower risks for the tumor. This confirmed observations made previously by other authors (90, 91). Although Dr. Correa’s model of carcinogenesis posits the start of CG with chronic gastritis which later progresses to atrophy and then to intestinal metaplasia-dysplasia-cancer in a sequential manner, whether or not atrophy precedes metaplasia remains unknown. The two could occur simultaneously. Verification of this sequence would only be possible if individual lesions could be followed prospectively without any intervention (92).

The concept of association between inflammation and GC had already been recognized by Virchow in 1863, when
he hypothesized that cancer originates in sites with chronic inflammation (93).

H. pylori infection stimulates both innate and acquired immune responses (94). The initial step in this process is recognition of the microorganism through Nod1 (nucleotide-binding oligomerization domain protein 1) which is an innate system for detecting bacteria which identify a Gram negative peptidoglycan muropeptides of these bacteria (94-97).

Immune system stimulation after H. pylori recognition by Nod1 results in chronic gastritis. The infiltrating inflammatory cells colonize the epithelium which influences colonization density, inflammation level and generation of adaptive immune response (94, 95). In this way the innate response is a key determinant of the severity of the disease and gastric carcinogenesis. This innate immune response against H. pylori, includes the release of antibacterial peptides and infiltration of the mucosa by all types of immune effector cells. Improper recognition of H. pylori by the innate immune system may contribute to the failure of the adaptive immune system to eliminate it. Certain polymorphisms of Toll-Like Receptor 4 3725 G/C have been found associated with increased risk of gastric atrophy (99) as well as GC (100). This indicates that this variation of innate immune system transmembrane protein recognizes pathogenic molecular patterns. Thus they are host factors involved in the response and outcome of infection. Besides the primary response, there is also an acquired humoral, local and systemic cellular immune response which persists throughout life. The response of the T cell is primarily Th1 (96, 97, 101, 102). This is the “wrong” answer, since H. pylori is an extracellular germ which, like similar microorganisms, should trigger a Th2 response (96, 97, 101, 102). The Th1 response produces interferon (IFN g), a tumor necrosis factor alpha (TNF a). IL-12, IL-18 (94-97). The polarization of the immune response towards Th1 with its cytokine profile may contribute to the development of more severe gastric pathology. In contrast, the activation of a Th2 response and the expression of cytokines such as IL-4, produce decreased gastric inflammation and protect against more severe pathologies, probably including GC. They counteract the effects of Th1 cytokines (96, 101, 102). These type I cytokines activate macrophages which in turn secrete proinflammatory factors along with adding bactericidal capacity, compared to activation by Th2 cell response (95-97). The severity of chronic gastritis is correlated with the number of cells that secrete IFN g (96, 62, 101, 102). The immune response differentiation towards Th1 seems to be influenced by the bacteria themselves and by environmental factors (62, 68, 96, 101). Parasitic infections which induce Th2 response have been suggested as one factor explaining the lower incidence of gastric cancer in regions of Africa with high prevalence of H. pylori infection (the “African enigma”) (103). However, this “enigma” has been challenged by some experts who believe it is really a myth since duodenal ulcers and GCs are both present in Africa (73). It was recently found that IL-17 and IL-23 are involved in H. pylori infection (96). The IL-17 cytokines produced by Th17 lymphocytes alter the immune surveillance and promote tumor growth (96). In China, Zhang et al (104) found that IL-23 and IL-17 were significantly elevated in patients with advanced GC, suggesting that Th17 cells may contribute to the carcinogenesis promoted by H. pylori.

When a person is infected with H. pylori, the GC risk is 2 to 3 times greater than for uninfected people, but if you have anti Cag A antibodies the risk increases to 11 times greater. Furthermore, depending on the degree of protein phosphorylation, this risk may increase further (105). If to all this is added the alteration of the gene that encodes synthesis of IL-1B-511, the risk increases to 87 times greater (68) (table 1). Modulation of the inflammatory process largely determines whether the outcome becomes neoplastic or not (60, 62, 68, 79, 70). A major difference between these two possibilities is acid secretion which, as mentioned, is normal in patients who have DU and increased in patients with gastric ulcer. Patients with body-antral gastritis generally have acid hyposecretion (62, 67, 70, 79). In the latter type of gastritis, host factors such as IL-1, IL-8 and matrix metalloproteinases, together with virulence factors of H. pylori protein Cag A, VacA and OipA, play an important role in the development of gastric ulcers (60, 107-110).

Gastric hyposecretion is partly determined by interleukin 1B (IL-1B). Besides being a proinflammatory cytokine, it is a potent inhibitor of acid secretion (100 times more potent than omeprazole) (111). Some investigators have found that polymorphisms of IL-1B gene and the antagonist of IL-1 (IL-1RN) are associated with both hypochlorhydria and increased GC risk (112-114), but others have
not found any association with CG (115-17). Three meta-

analyses have been conducted regarding this controversy. 

They have included multiple publications on the subject 

including analysis of IL-1B and IL-1RN polymorphisms 

(118-120). Two of them concluded that proinflammatory 

interleukin increases the risk of GC (118, 119), while the 

third concluded that it did not (120). The cause of this con-

tradiction is not known, although, given the multifactorial 

nature of GC it is conceivable that other genetic susceptibi-

lity factors, lifestyle factors and environmental factors may 

mitigate the effect of this particular polymorphism. 

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Risk: OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti <em>H. pylori</em> Ig G</td>
<td>2.2 (1.4 a 3.6)</td>
</tr>
<tr>
<td>Anti CagA</td>
<td>21 (8.3 a 53)</td>
</tr>
<tr>
<td>IL-1B511 + <em>H. pylori</em> CagA (+)</td>
<td>87 (11 a 679)</td>
</tr>
</tbody>
</table>

Despite the spread and growing support for the multiple 

step gastric carcinogenesis hypothesis, for some it is still 

questionable whether or not metaplasia is a premalignant 

condition. One reason for this is that diffuse gastric car-

cinomas, like signet ring carcinoma, do not appear to be 

associated with either atrophy or intestinal metaplasia. For 

this reason some experts think that these should be defi-

ned as paraneoplastic disorders instead of preneoplastic 

(121). However, more than a decade ago it was shown that 

intestinal types I and II metaplasia have no GC risks, but 

that type III has a relative risk of 4.6 (122). Furthermore, 

an 8 to 9 year follow-up study of 90 patients with type III 

intestinal metaplasia found that only one patient developed 

CG (123). Another more recent study by El-Zimaity et al 

(124), obtained similar results. They found no dysplasia 

or carcinoma in any of 33 patients with type II metaplasia 

and 34 patients with type III metaplasia during a 9 year 

follow-up. In Japan Kakinoki et al (125) recently found that 

the majority of gastric adenocarcinomas in patients infected 

with *H. pylori* developed when mild to moderate atrophy 

existed. These latest studies keep the controversy over the 

role of gastric metaplasia as a precursor of GC alive. Now 

there is evidence that preneoplastic and neoplastic cells, 

bone marrow derived progenitor cells (homing stem cells) 

in adults, have contributed to the chronic stomach inflam-

mation induced and perpetuated by *H. pylori* (126-128). 

These new findings do not discredit Correa’s model, but 

rather enrich it and highlight its importance. Different infla-

mmatory mediators are key to mobilizing the bone marrow 

derived progenitor cells and modulating the GC risk (fi gure 

3). Atrophy and metaplasia are permanent chronic infla-

mation indicators which are crucial for GC’s production 

of the three states of carcinogenesis: initiation, promotion 


![Figure 3. Outcomes of *H. pylori* infections.](image)

Gastric carcinogenesis 311
and progression (126-128). Inflammation induced by H. pylori involves complex molecular networks which are only partially known. They generate constant tissue destruction culminating in atrophy and intestinal metaplasia in genetically susceptible individuals when favorable environmental conditions exist. Initial repair is undertaken without success by stem cells from the peripheral blood. They are progressively replaced by bone marrow derived progenitor cells, the second line of defense, before the onset of severe ongoing inflammation and tissue destruction (82). Similarly, bone marrow derived progenitor cells could be the stem cell of gastric cancer. Given its high degree of plasticity it can give rise to many variants in different types of cells including those that form different structures of the tumor (129).

H. pylori DIRECT MECHANISMS (VIRULENCE FACTORS)

*H. pylori* is genetically more diverse than most other bacterial populations (130). This genetic diversity is considered to be a feature involved in this microorganism’s ability to produce different diseases. Since it is beyond the scope of this work to discuss the many virulence factors of this organism, only some which we consider to be the most relevant will be highlighted.

**The cag A Pathogenicity island (cag PAI)**

Most strains of *H. pylori* can be grouped into two distinct phenotypes based on the presence of the cag PAI (108, 110, 130, 131). The cag PAI is a new region of DNA acquired by *H. pylori* in the course of its evolution by horizontal transfer from other bacteria (131). This island contains 30 to 40 genes including the cytotoxic associated cag A, which encodes the Cag A protein (108, 130, 131). The cag PAI is present in 60% of *H. pylori* in Western countries and in more than 90% of the countries of Eastern Asia (107, 132). Cag A protein is highly immunogenic. It has a molecular weight between 128 and 140 kDa. The strains expressing this protein are more virulent than those that do not express it. (110,130-132). The first induce increased production of inflammatory cytokines such as IL-8, increased cellular proliferation and apoptosis (62, 107, 133). *H. pylori* injects these Cag A proteins into the cytoplasm of the epithelial cell by a Type IV secretory mechanism (molecular syringe) (134-136). Translocation of Cag A into gastric epithelial cells produce significant structural and functional changes that benefit bacteria (134). Once injected, the cell recognizes the protein as an epithelial signaling molecule. Similar to other signaling protein it is phosphorylated in varying degrees at sites that contain the Glu-Pro-Ile-Tyr-Ala sequence of five amino acids called EPIYA motifs (130, 134-136). When CagA is phosphorylated by these kinases it activates the tyrosine phosphatase SHP-2 Oncoproteins whose mutation is associated with malignancy in humans (130-136, 137.138). CagA deregulates SHP-2 to disrupt the Erk MAP kinase and FAK dephosphorylating focal adhesion kinases involved in inducing changes in cell morphology resulting in a highly elongated cell morphology termed the hummingbird phenotype (135-137). Cag A also damages cell-cell interactions independently of phosphorylation, destroys closed joints and causes loss of polarity in epithelial cells. It also destabilizes the E-cadherin / β-catenin complex (22, 130). Recently, the first experimental evidence that Cag A is real *H. pylori* Oncoprotein was obtained by demonstrating that in transgenic mice phosphorylation of Cag A is associated with tumors but not when it inhibited the ability to phosphorylate (139).

The higher the phosphorylation levels of Cag A protein the greater the potential for an oncogenic strain of *H. pylori*. Depending on phosphorylation site (EPIYA reasons), the Cag A protein may be one of two sub-types (130.139): Eastern Asian Cag A and Western CagA (130, 135). The EPIYA motif is part of four different EPIYA sites: EPIYA-A,-B,-C and D (138.139). Each site is determined by the amino acid sequence surrounding the sequence of EPIYA. In Western countries, the most common are Cag A EPIYA-A and B, followed by C sites, where C is repeated from one to three times (ABC, ABCC and ABCCC). The most common type is the ABC variant (135). In Asian countries most of the Cag A is EPIYA-A and D (ABD type). The importance of identifying these different types of Cag A lies in the different ways they are associated with GC prevalence and mortality. The Cag A of various regions of the world with high prevalence rates of GC are similar to East Asian Cag A. In contrast, where there is low prevalence of gastric cancer CagA is the Western type (130, 140).

In addition to the Cag A proteins, small effector molecules such as cell wall peptidoglycan are released into gastric cells by the type IV secretion system (141). In cell cytoplasm peptidoglycan is recognized by the innate immune system defense protein NOD1, leading to activation of nuclear factor k beta (NF-kB) which increases the expression of genes encoding pro-inflammatory proteins like IL-8, CXC chemokines and defensin B, an antimicrobial peptide (141).

**vacA and VacA**

VacA or vacuolating cytotoxin is a protein. It is one of the most important virulence factors of *H. pylori*. It is encoded by the gene vacA. It has a molecular weight of approximately 139 kDa and derives its name from one of its studied early effects, the production of massive vacuolization of gastric epithelial cells (110, 142-144). Unlike cagA, all strains
of *H. pylori* have vacA. However, its functional expression varies, so not all induce vacuolation of epithelial cells (110, 142, 143). While this gene is present in all strains of *H. pylori*, it has several polymorphisms (142, 143). Typically, it has been considered to have two regions: the “s” region or signal sequence region and the genome media region or “m”. For each there are two alleles: s1 and s2, and m1 and m2. All possible combinations of the two occur: s1m1, s1m2, s2m2 etc. The s1 and m1 in turn can be subdivided into s1a, s1b, s1c, m1a, m1b and m1c (143-145). Besides cellular vacuolization, VacA produces alterations in the pore formation of the gastric epithelium (110, 147), in alteration of close intercellular unions, in apoptosis, in suppression of the host’s immune system, in blocking macrophage phagosomes, in interference with antigen presentation to T cells, and in the inhibition of activation and proliferation of T lymphocytes (down regulation) (110, 146). This geno-
cells, and in the inhibition of activation and proliferation of phagosomes, in interference with antigen presentation to T
region i1. For this reason the authors have concluded that vacA (147).

### Other virulence factors

Besides the vacA and cagA *H. pylori* has many other virulence factors. Among them are the inflammatory outer membrane protein (OMP), Blood Group Antigen Binding Adhesin (BabA) and AlpAB adhesin (60, 107.110, 148 -151). BabA, is a foreign protein which allows *H. pylori* to adhere to Lewis B antigens expressed on the membranes of gastric epithelial cells (148). Strains expressing these proteins produce more severe lesions. The respective proteins encoded by these genes are closely related to *H. pylori* associated gastroduodenal diseases, including GC (73, 140). So far, no one knows the exact mechanism of action of *H. pylori*’s multiple virulence factors. Knowledge of them, their interactions and their genetic variation could help identify patients at increased GC risk (59, 73, 80). For example the interaction of CagA positive strains with the S11 polymorphism of IL-1B increases the risk of GC, while association of inflammatory OMP with cagPAI influences the levels of IL-8 since both are needed to activate the promoter region of these cytokines (150, 151). Other additional mechanisms by which *H. pylori* promotes carcinogenesis (152) are shown in table 2.

**Table 2. Oncogenic mechanisms produced by *H. pylori***

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive regulation of vascular endothelial growth factor Mutations of p53</td>
<td>Increased expression of inducible nitric oxide synthase</td>
</tr>
<tr>
<td></td>
<td>Increased expression of COX2</td>
</tr>
<tr>
<td></td>
<td>Increased synthesis of prostaglandin E2</td>
</tr>
<tr>
<td></td>
<td>IL-8</td>
</tr>
<tr>
<td></td>
<td>Increased expression of metalloproteinase 9</td>
</tr>
<tr>
<td></td>
<td>Atrophic gastritis</td>
</tr>
<tr>
<td></td>
<td>Recruitment of bone marrow derived stem cells</td>
</tr>
<tr>
<td></td>
<td>Apoptosis of T lymphocytes, causing immune evasion</td>
</tr>
<tr>
<td></td>
<td>Increased expression of Ki67</td>
</tr>
<tr>
<td></td>
<td>Injures cellular joints containing E-cadherin</td>
</tr>
</tbody>
</table>

Modification of Kontouras (ref 152).

### Host genetic factors

Genetic polymorphisms of the infected individual influence the various clinical manifestations of *H. pylori* infections. Among the previously mentioned polymorphisms, IL-1B and IL-1RN are associated with increased GC risk (118-120). Another independent GC risk factor is tumor necrosis factor alpha (TNFa) genotypes (153). TNFa is a cytokine with strong proinflammatory activity. It is also induced by *H. pylori* (8, 60, 93, 96, 108) Like IL-1B, but to a lesser extent, it also inhibits the production of HCl.

The A allele of this cytokine has an OR of 2.2 for CG (95% CI 1.4-3.7) (154). In contrast, IL-10 is an anti-inflammatory cytokine that produces negative regulation (down regulation) of proinflammatory cytokines (IL-1B, TNFa, interferon G etc). Its deficiency contributes to increasing the Th1 immune response, and with it increased gastric inflammation (80, 96, 152, 153). Other described polymorphisms that are associated with increased risk of GC are those of the IL-8 promoter region (155, 156).

---

Gastric carcinogenesis 313
Gastric cancer prevention

Taking into account the incontrovertible association of *H. pylori* with gastric cancer, eradication of these bacteria should be an excellent strategy for preventing this cancer. However, the efficacy of this strategy has yet to be demonstrated. Currently it is agreed that the optimum moment for eliminating the microorganism and diminishing the risk of gastric cancer is prior to the development of atrophy and intestinal metaplasia (157-159). This was demonstrated by Wong in China (158). Recently a case was reported of two patients in whom *H. pylori* was eradicated after intestinal metaplasia and atrophy had developed who later developed gastric cancer (160). For this reason the development of after intestinal metaplasia and atrophy can be considered as the point of no return for the development of gastric cancer. Eradication of the bacteria after this point does not diminish the risk of gastric cancer (158-160). The implication is that the eradication of *H. pylori* does not prevent gastric cancer in all patients, and when these advanced gastric lesions exist, endoscopic follow up is necessary for early detection of gastric cancer (160). On the other hand, for patients with gastric MALT lymphoma, eradication of *H. pylori* cures the lymphoma in the majority of patients (161).

Recently a transcendental advance in the prevention of *H. pylori* was achieved in a double blind phase I study undertaken by Malfertheiner and colleagues (162). They found a prophylactic recombinant vaccine which uses VacA, CagA and the neutrophil activating protein of *H. pylori* (NAP-HP) to induce antibody production. The vaccine was successful in 86% of the healthy volunteers who participated in the study. Their ages were between 18 and 40. The authors’ conclusion is that this combination of antigens has an acceptable level of safety and immunogenicity and stimulates memory T and merits additional clinical study.

**CONCLUSIONS**

There are two pathways involved in *H. pylori* carcinogenesis. One is through indirect mechanisms of inflammation induced by persistent infection accompanied by hyper proliferation of cells with a high risk of DNA damage by free radicals. Bone marrow derived stem cells are part of this process and could be the stem cells of gastric cancer. The second route involves direct actions of *H. pylori* proteins on gastric cells. In the light of available information it is likely that both pathways are involved in the genesis of gastric cancer (figure 4). Among the host’s genetic factors there is evidence that genetic polymorphisms of IL-1B, TNF, IL-8, gama INF and IL-10 among others induce strong inflammatory responses that are associated with increased GC risk. Finally gastric cancer is a multifactorial disease involving genetic factors of the individual, environmental factors and most importantly, *H. pylori* infection (figure 5).

**Figure 4.** *H. pylori* and carcinogenesis pathways.
Figura 5. *H. pylori* and gastric cancer (agent, host, environment).

**REFERENCES**

42. Liu BH, Lee YK. Effect of total secondary carotenoids extracts from Chlorococcum sp on Helicobacter pylori-infected BALB/c mice. Int Immunopharmacol 2003; 3: 979-86.
50. Mirvish SS. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyn-


117. Lee SG, Kim B, Choi W, Lee I, Choi J, Song K. Lack of association between pro-inflammatory genotypes of the interleukin-1 (IL-1B -31 C/+ and IL-1RN *2/*2) and gas-


